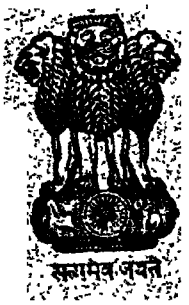

ANNUAL REPORT

OF THE

CENTRAL RESEARCH INSTITUTE, KASAULI

FOR THE YEAR

1962

CONTENTS

I. STAFF

Director

1. Dr. J. B. Shrivastav, M.D. (Bombay), Dip. Bact. (London).

Assistant Directors

*1. Dr. C. B. D'Silva, M.B., B.S. (Madras).
2. Dr. E. K. Narayanan, M.A., M.Sc., Ph.D. (Madras).
3. Dr. A. K. Thomas, M.B., B.S. (Bombay) Dip. Bact. (Manchester).
4. Dr. J. C. Suri, M.B., B.S. (Punjab), M.Sc. (Melbourne).
5. Dr. S. C. Agarwal, M.B., B.S., M.D., Ph.D. (London).
†6. Dr. A. L. Bhatia, B.Sc., M.B., B.S. (Punjab).
7. Dr. P. S. Menon, M.B., B.S. (Madras).

Deputy Assistant Directors

1. Dr. (Miss) P. Devi, B.A., L.T., M.Sc., (Madras), Ph.D. (Glasgow)
2. Mrs. Jasbir Kaur, M.Sc., w.e.f. 22-6-1962.

Medical Assistants

‡1. Dr. A. N. Rai Chaudhuri, M.B., B.S. (Calcutta).
2. Dr. S. C. Chatterjee, M.B., B.S. (Calcutta) D.T.M. (Calcutta).
3. Dr. M. L. Nath, L.M.F. (Calcutta).

Veterinary Assistant Surgeon

1. Sh. Rajan Bir Singh, B.V.Sc. and A.H.

Assistant Accounts Officer

1. Sh. Karam Singh, B.A., S.A.S.

Administrative Officers

1. Sh. Sangat Singh, B.A. upto 12-8-1962.
2. Sh. P. N. Sadhoo, B.A from 13-8-1962.

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*On deputation to B.C.G. Vaccine Laboratory, Guindy, Madras, as Director.

†On foreign service with I.C.M.R.

‡On deputation to All India Institute of Hygiene and Public Health, Calcutta, as Medical Officer for Clinical Pathological Service.

II. HISTORY, EXPANSION PROGRAMME, FUNCTIONS, ETC.

Short History

The Central Research Institute was established at Kasauli in 1905.

For the first time in the country the Institute introduced in 1906 two important biological products—a serum for treatment of snake bites and a vaccine for the prevention of typhoid fever. The Institute carried out survey on malaria and *kala-azar* in the country from 1906 to 1912, and it started functioning as a diagnostic laboratory for infectious diseases in the country. The Institute started manufacturing Cholera vaccine from the year 1914.

Research carried out during the period from 1926 to 1932 filled many gaps in the knowledge of malaria, kala-azar, relapsing fever, medical entomology, plague, cholera and treatment of snake bite. In 1939, the Pasteur Institute at Kasauli which formed a nucleus for the manufacture of Antirabic Vaccine prior to 1905 was merged alongwith the Drumbar Estate with the Central Research Institute. The Institute started functioning as a Research Laboratory and a Centre for distribution of standard sera.

During the Second World War a special unit was raised at the Institute for the vaccination and assay of Yellow Fever Vaccine. Work in mammalian Malaria, immunological studies on cholera and behaviour of Shiga toxin on monkeys continued from 1944 to 1946.

In 1948, the Microfilm and Photocopy Unit of the Indian Research Fund Association (now Indian Council of Medical Research) was established at the Institute.

In 1953, the Institute undertook a training programme for the diagnosis, prevention and treatment of rabies. Now a course is held every year in the month of April for a period of three weeks and facilities exist for training 20 medical and veterinary officers deputed by State Governments and other public health bodies all over the country.

In 1958, a Field Unit was established at the Institute to carry out laboratory investigations of outbreak of bacterial and viral diseases occuring in north-western part of the country. A National Salmonella and Escherichiaea Centre was also established with the co-operation of the World Health Organisation to collect and type the different bacterial strains belonging to these two groups of micro-organisms.

An Influenza Centre has been established at the Central Research Institute with the following objects:—

(i) Studies in influenza virus in areas of Delhi, Punjab and Uttar Pradesh.

(ii) Manufacture of Influenza vaccine.

In 1959, the Institute was recognised for Post-graduate training i.e. M.D. (Pathology and Bacteriology) Ph. D. (Pathology and Bacteriology) Ph. D. (Biochemistry) by different Universities of India.

Since 1961, the Institute is conducting B. Sc. (Hons) course in Microbiology to which only Institute employees having three years service and otherwise eligible are admitted.

In 1961, a new section viz. Triple Vaccine Section was formed for the manufacture of Diphtheria, Tetanus and pertussis Vaccine at the Institute, in collaboration with World Health Organisation and UNICEF who provided equipment, glassware, chemicals etc.

Expansion and Future Programme of the Institute

1. *Establishment of the Research & Training Wing at Chandigarh*—It has been decided by the Government of India to separate research and training from the manufacturing activities of the Institute so that both can develop unhampered with mutual advantage of each other.

At Chandigarh, one full sector consisting of about 240 acres of land has been offered free to the Institute by the Government of Punjab. It is proposed to build at Chandigarh, Research & Training Laboratories; a Biological Standardization, testing and Quality Control Laboratory; an Animal House for maintaining animals for the needs of the Institute and for other laboratories in the country; and a Farm for maintaining horses in more natural conditions and at a cheaper cost. The development of this Research and Training Wing at Chandigarh will be a phased programme and will extend over the 3rd and 4th Five Year Plans.

2. *Triple Vaccine*—A phased programme for the production of Diphtheria, Pertussis and Tetanus Vaccine has been drawn to reach the target of 7—10 million doses till third stage and 30—50 million doses in the 4th stage as detailed below:—

Phases	Annual production	To be completed in	
1st stage	One million doses each of Diphtheria, Tetanus and Pertuss Vaccines.	1964-65	3rd Five Year Plan
2nd stage	2-3 million doses each of Diphtheria, Tetanus and Pertussis Vaccines.	1965-66	
3rd stage	7-10 million doses each of Diphtheria, Tetanus and Pertussis Vaccines.	1967-68	4th Five Year Plan.
4th stage	30-50 million doses each of Diphtheria, Tetanus and Pertussis Vaccinies.	1970-71	

Preliminary work for the 1st stage of the Project with regard to planning, procurement of machinery, recruitment and training of technical staff etc. has reached an advanced stage, and if given the necessary additional assistance, the project can reach the targets set for the 1st stage of Production during the year 1964-65, and the 2nd stage of production during the year 1965-66. The building part of the Triple Vaccine project has been included in the Third Five Year Plan as a Plan scheme. The building programme consists of extension of the existing Anaerobic Block to provide for the laboratories for the manufacture of Triple Vaccine and an Animal Holding House.

3. *Lyophilisation of plasma*—To meet the national emergency, a separate unit is being established at the Institute for lyophilization of human plasma. For this purpose, blood is being received from the various blood collecting centres in the States of Punjab and Himachal Pradesh.

4. Construction Programme –

I. *Serum Concentration Block*—There is at present no separate building for Serum Concentration work. With the expansion of the Institute's activities in the direction of production of large quantities of Anti sera, it has been decided that a separate block should be constructed for Serum Concentration work. This block will be constructed on the property 'widdicomb' acquired from the Government of Punjab

II. *Additional Stables & Syce Quarters*—This Institute has to maintain horses for the manufacture of sera. With the proposed expansion in the manufacturing activities and to step up the output of antivenene, it is considered necessary to raise the number of horses to 100. Before the number of horses is increased, it is necessary to construct additional stables and Syce Quarters on the open land attached to the EAC's Bungalow.

III. *Animal Breed ng House*—For the proper maintenance upkeep and breeding of laboratory animals required for research and manufacturing purposes, a large animal house with modern arrangements is required. It is proposed to construct the Animal Breeding House on the flat land available in 'Widdicomb'.

IV. *Extension to the Anaerobic Block*—The existing building 'Anaerobic Block' is being modified for providing a full fledged laboratory for the large scale manufacture of Triple Vaccine at this Institute.

5. *Efforts of the CRI to meet the national emergency*—About 500 officers and men of the Central Research Institute, Kasauli contributed their one day's pay amounting to approximately Rs. 1600/- to the National Defence Fund. In addition, some gold ornaments have also been donated by the wives of the Institute employees.. They have further decided to work longer hours to meet the national requirements of sera and vaccines. The profits earned by the Co operative Society of the Institute have also been donated for the National Defence Fund. The Welfare Fund of the Institute donated a sum of Rs. 500/- towards the purchase of wool for knitting garments for the Jawans. Several employees of the Institute have volunteered for recruitment in the Army and for donating blood. Some reservists working at the Institute rejoined the Army and the Institute has agreed to look after the families of these reservists.

6. *Rifle training*—Under the Rifle Training Scheme of the Punjab Home Guards, four batches each consisting of 20 employees of the Institute underwent rifle training. More batches are being sent for training under the above scheme.

Functions

The major functions of the Institute are as follows—

(i) Research, both basic and applied of medical and public health importance.

(ii) Maintenance of large collections of 'Type' cultures of pathogenic organisms for use of research workers in the country, and to act as National Centre of Salmonella and Escherichiae groups of organisms.

(iii) Carrying out of laboratory diagnostic work for private practitioners, government and non-government institutions.

(iv) Receiving and distributing cultures for international standards of toxins and anti-toxins and serving as a recognised centre for yellow fever vaccine.

(v) Manufacture of biological products, such as TAB vaccine, cholera vaccine, antirabic vaccine, diphtheria toxoid, tetanus antitoxin, tetanus toxoid, antivenom serum, antirabic serum and influenza vaccine etc.

(vi) Serving as the Government of India's Central Drug Laboratory in respect of all biological products, imported or manufactured in the country, in accordance with the provisions of the drugs Act, 1940.

(vii) Information Bureau, by supplying expert advice on questions concerning rabies, snake bite, cholera, typhoid and other communicable diseases and also to advise the Government of India, Indian Council of Medical Research and Indian Pharmacopoeia Committee on these matters.

(viii) A training centre for laboratory personnel in techniques for bacteriology, virology, immunology and biochemistry and for medical and veterinary personnel in diagnosis, prevention and treatment of rabies. The Institute conducts regular classes for B.Sc. (Hons) in microbiology of the Punjab University and also imparts training for post-graduate studies in pathology, bacteriology and biochemistry for which it has been recognised by several Universities.

Pattern of Organisation and Management

The Institute has the following six main sections—

(1) Bacterial Vaccine Section (Includes kitchen, media, bottling and ampouling sections).

(2) Biochemistry Section.

(3) Biological Standardization and Quality Control Section.

(4) Serum Concentration Section.

(5) Triple Vaccine Section.

(6) Virus Section.

In addition to these six major technical sections there are the following eleven other sections each in the charge of a qualified and experienced member of the staff:—

(1) Accounts and Cost Accounts Section.

(2) Administration Section (General and Office).

(3) Clinical Pathology and Bacteriology.

(4) Editorial office of the Indian Journal of Medical Research.

(5) Field Unit.

(6) Libraries (Central Research Institute and Indian Council of Medical Research).

(7) Maintenance and Engineering (Workshop).

(8) Microfilm Unit (Indian Council of Medical Research).

(9) National Salmonella and Escherichiae Centre.

(10) Stables and Animal House.

(11) Stores.

Publications by the Officers of the Institute during the year 1962:

1. Dr. J.C. Suri ..	1. Passive Immunization against Tetanus with human immune Globulin	British Med. Journal, Vol. ii, 79 81, 1962.
	2. Triple Vaccine for children ..	Swasth Hind. Vol. VI, 107, 1962.
2. Dr. S.C. Agarwal	1. Classification and nomenclature in Family Enterobacteriaceae.	Ind. Jour. Med. Res. 50, 541—544, 1962.
	2. Salmonella Serotypes identified at National Salmonella and Escherichia Centre, Kasauli in 1958—60.	Ind Jour. Med. Res. 50, 567-568, 1962.
	3. Pathogenisis in diarrhoeal disease in relation to entero-pathogenic coli.	Ind Jour. Med. Res. 50, 593—598 1962.
	4. Isolation of Salmonella Singapore from a case of infantile enteritics.	Ind. Jour. Med. Res. 50, 690 691 1962.
	5. "Chloromycetin Resistance of Salmonella species in India" 1958—61.	Bull. Wld. Hlth. Org. 27(3) 331—335, 1962.
3. Dr. A.L. Bhatia	1. The rate of inactivation in South Indian patients with pulmonary tuberculosis. I. The microbiological assay of isoniazid in serum following a standard intramuscular dose.	Bull. Wld. Hlth. Org. 25, 765, 1961. Reprinted in Indian J Tuber. 9, 111.
	2. The response of patients infected with isoniazid resistant tubercle bacilli to treatment with isoniazid plus PAS or isoniazid alone.	Bull. Wld. Hlth. Org. 25, 807, 1961
	3. Virulence in the guinea pigs of isoniazid-sensitive tubercle bacilli isolated from South Indian patients before and after 3 months of chemotherapy.	Bull. Wld. Hlth Org. 25, 759. 1961 Reprinted in Indian J. Tuberc 9, 45.
	4. Addition of Catalase to Lowenstein Jensen medium.	Tubercle (Lond.) 42, 537, 1961.
	5. Catalase-enriched medium ..	Amer. Rev. Resp. Dis. 85, 133, 196
	6. The clinical implications of the heterogenity of the virulence in the guinea-pigs of cultures of tubercle bacilli from South Indian patients.	Proceedings of the Eighteenth Tuberculosis Workers' Confer ence, 1962. Tuberculosis Association of India *In press.*
	7. The Course of pulmonary tuberculosis in patients excreting organisms which have acquired resistance to Isoniazid; response to continued treatment for a second year with isoniazid alone or with isoniazid plus PAS	Bull. Wld. Hlth. Org. 26, 1, 1962.
	8. The growth rate of India tubercle bacilli.	Ind. Jour. Med. Res. 50, 33 1962.
4. Dr. (Miss) P. Devi	1. Endemic Fluorosis (An epidemiological, biochemical and clinical study in the Bhatinda District of Punjab).	Ind. Jour. Med. Res. 50, 387-39 1962.

Visits, Training Etc.

1. Dr. J. B. Shrivastav, Director, attended the meeting on "Field and Laboratory Studies of Cholera Vaccine" from 30th November to 8th December and the meeting of the W.H.O. Expert Committee on Biological Standardization from 10th to 15th December, 1962, held in Geneva.

2. Dr. M. L. Nath, Medical Assistant, C.R.I., Kasauli, received training in production, assay & testing of Bacterial vaccines, toxins, toxoids, anti-toxins and high titre sera at Copenhagen (U.K.) from 9th March 1962 to 31st July 1962 under a W.H.O. Fellowship.

Training

Nine Doctors and Research workers from India and abroad received practical training at the Institute in the Laboratory techniques for production and research during the year, 1962.

Commercialisation of Accounts

Revision of Sale-rates—The sale-rate of one of the major products i.e. Antirabic serum has been revised by the Government of India during the year according to its cost of production. The Director has now been delegated the power to fix, on an ad hoc basis, the selling rates of the newly manufactured biological products till their manufacture starts on a commercial scale. Accordingly the sale-rates of Avianised Rabies Vaccine, Schick Test Toxin and Distilled water have been fixed on ad hoc basis.

Proforma Accounts—During the year 1962, certain changes in the previously approved forms of the Proforma Accounts were intimated by the Accountant General, Punjab, Simla for adoption from the year 1961-62. The Proforma Accounts for the year have been prepared in the revised form. After these accounts have been finally checked by the Director of Commercial Audit, New Delhi, corresponding changes will be made in the approved Accounts Rules of the Institute.

Budget

(a) The Institute is financed by the Central Government and the budget grant for the year 1961-62 was Rs. 12,20,800 (Non-plan) and Rs. 21,000 (Plan) in respect of its manufacturing and research activities. The sub-headwise details of expenditure of Rs. 12,91,420.89 nP incurred during this financial year are as under:—

	Rs. nP.	Rs. nP.
(i) Pay of Officers	.. ..	1,07,973·25
(ii) Pay of Establishment	.. ..	3,99,671·91
(iii) Allowance and Honoraria etc., T.A. including P.T.O. concession	6,625·01	
Dearness Pay/Dearness Allowances	53,325·78	
H.R.A. and other allowances	56,332·98	
		1,16,283·77
(iv) Other Charges—(Purchase of Serum bottles, Purchase of chemicals, dyes, etc., Purchase and Repair of Apparatus, Purchase, upkeep and food of animals, Miscellaneous contingencies and entertainment charges)		6,67,491·96
Total ..		12,91,420·89

(b) Receipts from the sale of Sera and Vaccines etc. manufactured at the Institute were Rs. 11,19,339.50 nP only.

III. RESEARCH WORK DONE IN 1962

1. *Bacterial Vaccine Section*

(1) *Polyvaccine against cholera and typhoid group of infections (In I.C.M.R. Enquiry under Shrivastav J.B.)*—The enquiry commenced from 1st April, 1960 with the object of preparing improved vaccines against cholera and typhoid group of infections. It was envisaged to prepare vaccines with the addition of mineral oil adjuvants and inorganic adsorbing depot agents, first singly and then in combination, for both cholera and typhoid, and later to extend the work by combining these with chemically fractionated antigens. In view of the encouraging reports on combination of antigens at the Soviet Research Institute of Serology & Immunology (1915) and in view of the fact that the duration of response of cholera vaccine is rather short, it was considered advantageous to investigate the possibility of preparing a single dose combined depot vaccine with a prolonged immunizing effect.

The preliminary work of preparing bacterial emulsions and high titre anti-sera against cholera Inaba, Ogawa and rough 'O' antigens, and for Salm. typhi (O, H & Vi) and Salm. paratyphi A and B (O & H) which was undertaken in the previous year, was further extended. While Roschka's method of treatment with alcohol and acetone proved useful for preparing antisera for Salm. typhi and Salm. paratyphi A and B, the alcohol and acetone treatment was not found of advantage for preparing the cholera anti-sera.

Another pre-requisite was suitable challenge strains for the animal protection tests. Thus 8 strains of *V. cholerae* were tested in a similar number of experiments and 7 strains of *Salmonella. typhi* were tested in 10 experiments. Those strains which were found suitable were selected.

Cholera

(i) *Agglutination response of various cholera vaccines*—Table I gives the various adjuvant and depot vaccines prepared and used for this purpose.

TABLE I

Vaccine	Adjuvants or inorganic substances added and their proportion	Total No. of Organisms/ml	Dose to be injected
1. Control Cholera vaccine	Nil	8,000 million organisms Inaba & Ogawa in equal proportion.	Two doses of 0·05 ml and 1 ml at an interval of one week injected intramuscularly.
2. Cholera vaccine with Falba & Bayol F.	Falaba. Bayol F Concentrated Vaccine 1ml 9 ml 10ml	24,000 million organisms/ml	0·5 ml intramuscularly.
3. Cholera vaccine with Arlacel A & Bayol F.	Arlacel A Bayol F Concentrated Vaccine 1 ml 9ml 10 ml	24,000 million organisms/ml	0·5 ml intramuscularly

TABLE I—*contd.*

1	2	3	4
4. Cholera vaccine with Arlacel A & almond oil.	Arlacel A 1ml Almond oil 9ml Conc. Vaccine 10 ml	24,000 million organisms/ml	0·5 ml intramuscularly.
5. Cholera vaccine with liquid paraffin & lanolin.	Liquid paraffin 10ml Lanolin. 1 ml Concentrated vaccine 15 ml	24,000 million organisms/ml	0·5 ml intramuscularly
6. Cholera vaccine with calcium phosphate.	The final 0·5 ml dose of vaccine contains 3 mgm calcium phosphate.	24,000 million organisms/ml	0·5 ml intramuscularly.
7. Cholera vaccine with aluminium phosphate.	The final 0·5 ml dose of vaccine contains 1·5 mg. aluminium phosphate.	24,000 million organisms/ml	0·5 ml intramuscularly.

21 rabbits of approximately 1500—1800 gms. weight were taken and each vaccine was injected intramuscularly in the dose shown in the table in 3 rabbits. A random procedure was adopted while injecting the various vaccines. While setting up the agglutination tests and reading the results, the identity of the vaccine was not revealed.

Thus in the case of each vaccine 12,000 million organisms were injected in each rabbit. The blood was collected from each rabbit at weekly intervals in the first month and at monthly intervals thereafter. For each vaccine the geometric mean of the agglutinating titre of the 3 rabbits was taken.

The results are being analysed.

(ii) *Mouse protection tests*—

(a) *Active immunization of mice*—A method of assay was developed which yielded reproducible results. In this active immunization of mice was done with six graduated doses arranged in a geometrical progression so that the 50% protection dose was about the middle of the selected series. The selected doses were given *subcutaneously* in 2 equal parts with an interval of 7 days between them. This was followed 10 days later by a test infection with a virulent strain of *V. cholerae*. The total volume of challenge dose was equal to 0.5 ml in 5% mucinized suspension.

The vaccines were tested against both Inaba & Ogawa standard test infection doses separately and the mice were observed for 72 hours. The 50 per cent end point was calculated by the method of Reed & Muench (1938) and the relative potencies of the vaccines mentioned in Table I was determined.

(b) *Passive immunization of mice*—Immune sera were prepared in rabbits. Six graduated doses in geometric progression of the immune serum prepared against each vaccine were given intraperitoneally 4 hours before challenge in a volume of 0.2 ml followed by a challenge also given intraperitoneally in a volume of 0.5 ml in 5 per cent mucinized suspension, and representing the same number of organisms as used for active immunization test. The mice were observed for 72 hours and the dose of serum

determined, which would protect 50 per cent of the mice. Separate observations were made after challenge with Ogawa and Inaba infective doses.

(c) *Assessment of vibriocidal titre after immunization with two doses of vaccine*—Guinea-pigs 300-400 gm. in weight were given 2 doses of vaccine (0.5 ml and 1 ml) at weekly intervals subcutaneously and the vibriocidal titre tested *10 days after the second* immunizing dose by the method described by Ahuja and Singh (1948).

(d) *Serological examination of vaccines using mono-specific Ogawa, Inaba and Cholera rough 'O' Sera*—Agglutination results of various vaccines were determined and the comparison done with regard to the titre against mono specific cholera 'O' sera and cholera rough 'O' sera.

Typhoid

The initial investigations have been completed and the work is in progress on the typhoid vaccines made with adjuvant and depot agents.

(i) *Agglutinin response in rabbits for H., O & V. agglutinins*—Table 2 shows the adjuvants & depot vaccines prepared and used for this purpose.

TABLE 2

Vaccine	Adjuvants or inorganic substances added and their proportion		Total number of organisms/ml.	Dose to be injected
1. Control Vaccine ..	Nil		1,000 million organisms/ml (Rawling strain and Vi strain of Salm. typhi in equal proportion).	Two doses of 0·5 ml and 1 ml at interval of 1 week injected intramuscularly.
2. Typhoid vaccine with Falba & Bayol F.	Falba Bayol F Concentrated vaccine	1 ml 9 ml 10 ml	3,000 million organisms/ml	0·5 ml intramuscularly.
3. Typhoid vaccine with Arlacel A and Bayol F.	Arlacel A Bayol F Conc. vaccine	1 ml 9 ml 10 ml	3,000 million organisms/ml	0·5 ml intramuscularly.
4. Typhoid vaccine with Arlacel A & almond oil.	Arlacel A Almond oil Conc. vaccine	1 ml 9 ml 10 ml	3,000 million organisms/ml	0·5 ml intramuscularly.
5. Typhoid vaccine with liquid paraffin and lanolin.	Liquid paraffin Lanolin Conc. vaccine	10 ml 5 ml 15 ml	3,000 million organisms/ml	0·5 ml intramuscularly.
6. Typhoid vaccine with calcium phosphate.	The final dose of vaccine contains 3 mgm. calcium phosphate.	0·5 ml	3,000 million organisms/ml	0·5 ml intramuscularly.
7. Typhoid vaccine with Aluminium phosphate.	The final dose of vaccine contains 1·5 mgm. of aluminium phosphate.	0·5 ml	3,000 million organisms/ml.	0·5 ml intramuscularly.

The methods employed have been the same as described for cholera vaccine. The formation of antibodies was determined weekly during the first month and at monthly intervals thereafter. For these tests the H and O agglutinating suspensions were prepared from formalinized broth cultures

of strains H 901 and 0961 respectively. The Vi agglutination was done with living suspensions of Vi (Bhatnagar) strain. The culture was so diluted with normal saline that it matched with Brown's Opacity tube No. 3 and one drop of the concentrated bacterial emulsion was used as the suspension.

(*ii*) *Mouse protection tests*—

(a) *Active immunization of mice*—The vaccines were tested at 3 dose levels viz. 100 million: 10 million and 1 million organisms. The subcutaneous route of immunization was preferred to the intramuscular (Felix, 1951). Two immunizing doses were given at weekly intervals. The challenge dose of 0.5 ml suspended in saline of TY2 strain and containing 1,000 million organisms was given intraperitoneally 7 days after the second immunizing dose.

(b) *Passive immunization of mice*—The immune sera against the different vaccines was prepared in rabbits. 0.5 ml of the following dilutions of the pooled sera were injected subcutaneously, undiluted, 1:2, 1:4, 1:8, 1:16. The challenge dose used was the same as for the active protection tests and was given 48 hours after serum administration.

Chemical Fractionated Antigens

With a view to assess the immunizing response of the chemically fractionated antigens from cholera and typhoid group of organisms the following work was done during the period under review—

Cholera—Immunologically potent components, polysaccharide in nature, have been prepared from *V. cholerae* by the method of Felton *et al* (1945). This method of co-precipitation with calcium phosphate was originally employed for isolating antigenically active polysaccharides from pneumococcus. The product obtained affords a high degree of protection in mice and gives precipitin titre in high dilution (1:100000) against antiserum from *V. cholerae*. The animals (mice) were immunized by administering subcutaneously 0.2 mg of the substance at weekly intervals and protection determined by challenging them 10 days after the 2nd dose with mucinised suspensions of live *V. cholerae* cells. As far as toxicity of the samples is concerned the minimum lethal dose was 1.75 mg. This shows that the toxicity dose is approximately 4 times more than the immunising dose. However, it has been felt necessary to pool the polysaccharide prepared from a few batches and after test on laboratory animals, the pooled preparation if found to give satisfactory immunizing response will be put to field trials in human beings.

It is proposed to fortify the present cholera vaccine with the polysaccharide fractions, incorporate adjuvants and depot agents and test it for its immunological behaviour in vivo.

Typhoid—To obtain antigenic fractions from typhoid group of organisms preliminary trials have been made to isolate these by Raistrick and Topley's method (1934) of digesting the bacteria with trypsin. This fraction will also be incorporated with the cellular typhoid vaccine to see if it gives better immunity and if it can replace or supplement the antigens in the adjuvant and depot vaccines.

2. *Sterility testing of vaccines* (*Bhatia A.L. & Nath M.L.*)—Experiments were set up to observe the effect of inoculum size, number of samples taken for testing, and the nutrients present in the medium, on the efficacy of the tests to pick out the contaminated batches of biological products.

3 *Bacteriological examination of water supplied in the adjoining hilly areas and the effect of environmental factors (Parkash Singh & Bhatia A.L.)*—The bacteriological purity of nearly twenty sources of water supplies used for drinking purposes in the adjoining hilly areas and the effect of factors like rainfall is being studied.

4. *Differential analysis of strains of mycobacteria. (Bhatia A.L.)*—Work is in progress on the differential analysis of strains of mycobacteria obtained from Indian patients, especially with regard to the colonial morphology, growth at 23°C, pigmentation after exposure to light, catalase activity, drug sensitivity, neutral red test and the niacin activity.

Detailed study was done on four strains isolated from extra-pulmonary lesions with a view to type them. Further, an attempt is being made to correlate the sensitivity of the organisms, isolated from patients before the start of treatment, to their response to chemotherapy.

2. *Biochemistry Section*

Immunochemical Enquiry with reference to vibrio-polysaccharides and protective role of these factors in cholera immunity (An ICMR Enquiry under Shrivastav J.B.)—Polysaccharide fractions obtained by adsorption on calcium phosphate following Felton *et al* (1935) method were throughout biuret-negative, glycogen-negative and showed a low nitrogen content. They were devoid of ribose indicating thereby that they are free from intracellular material like nucleic acid and since they were immunologically more potent than the fractions obtained by other methods, this method was selected as the method of choice. It was felt necessary to check the reproducibility of the method and therefore 12 batches of polysaccharide have been prepared by growing the same strain of *V. cholerae* Inaba 569 B in papain-broth. The autolysed culture was processed under the same controlled conditions for the isolation of the polysaccharide. The product in each case was subjected to chemical as well as immunological analysis. No significant variation in chemical values was noticed from batch to batch. The results in brief are given below:—

The total nitrogen content varied from 1.34—2.74%; Reducing sugar from 55.27—67.87%; Phosphorus from 0.56—0.87%; Hexosamine from 15.10 19.76%; Pentose from 9.73—14.98% and Glucuronic acid content varied from 15.63 to 21.13%. The sugar constituents as identified by chromatography of the acid hydrolysate of the polysaccharide preparations showed presence of arabinose, xylose, glucose and galactose along with amino sugar as glucosamine. This method was compared for reproducibility in analytical values with other methods so far tried, for the isolation of the polysaccharide and it was found that in this respect the method of Felton *et al* (1935) was better than any other method.

Fractions from *V. cholerae* by co-precipitation method showed high precipitin titre against homologous serum and were immunologically active in vivo. In two cases where the animals (mice) were immunised with graded doses, the Effective immunising dose (ED 50) was found to be 0.01 mg. Fractions from NAG, El Tor and Rough vibrios were also tested for precipitin activity against homologous sera and serum raised against *V. cholerae*. Although they all gave positive reaction with the sera raised against their own types, they did not cross react with cholera serum, proving thereby the serological specificity of the fractions.

The method of co-precipitation with calcium phosphate satisfied all the criteria laid down for a suitable method with the only drawback that it did not give a high yield of the polysaccharide. With a view to improve the yield, 3-day continuously shaken autolysed cultures were employed. In three batches prepared so far although the growth of the organism was abundant (3 times than the growth from unshaken cultures), the yield of the polysaccharide did not increase proportionately. On chemical analysis it was found that the product contains a higher lipid content than from the one usually obtained from stationary cultures. However, the fraction when tested in vivo (mice) showed same degree of protection as shown by polysaccharide preparations from unshaken cultures and hence the quantitative change in chemical composition was not reflected in the antigenic behavior. This interesting observation is being confirmed by working with more batches of autolysed cultures shaken for 72 hours.

More studies have been carried out on the chemical nature of the preparations at hand. It may be mentioned that the antigenic complex seems to contain 8—10% of lipid moiety thus showing that the complex may be lipo-polysaccharide in nature. Since it appears that lipid is an integral part of the antigenic complex, a method is being standardized for the the estimation of this constituent in micro amounts. Enzymic degradation of the fraction has also been taken up. The degraded fractions will be tested immunologically and results compared with the undegraded ones.

To overcome the difficulty of handling large bulks of liquid media giving low yield of polysaccharide, growth of vibrios on solid media (Papain-agar) is being tried. Eighteen hours growth is scrapped and suspended in sterile saline to the opacity comparable approximately to a 3-day autolysed broth culture. Autolysis of this suspension is done for 72 hours, with occasional shaking of the cultures during incubation. Polysaccharide is then isolated by adsorption on calcium phosphate as in the original method.

So far two batches of polysaccharides have been prepared following the above procedure. In one case starting with 10 grams of wet bacteria the yield of polysaccharide was 0.1 gm and in the second case starting with about 30 gms of wet bacteria the yield was 0.25 gms. The preparations were Molisch-positive and biuret-negative. These preliminary trials show that the yield is still low. It appears that the co-precipitation method cannot be applied successfully in the case of bacteria obtained from solid media.

Standardisation of the method of co-precipitation with calcium-phosphate—Since co-precipitation of the polysaccharide on calcium phosphate has been selected as a method for extracting antigenically active polysaccharide from *V. cholerae* it was considered essential to standardise this method. For this purpose, adsorption of the polysaccharide on calcium phosphate formed *in situ* at different pH's is being tried to find out an optimum pH at which the adsorption may be maximum, thus giving a higher yield of the polysaccharide. However, it has been kept in mind that such a fraction should not show a nitrogen content higher than 1-3%, thereby affecting the purity of the product. So far adsorption at three different pH's have been tried. It has observed that at pH 10.6 the yield of the polysaccharide is almost three times than obtained usually at pH 9. On chemical analysis this fraction did not show much variation in analytical values from other preparations. Antigenic behaviour of the product in

vitro and in vivo is satisfactory. More batches of polysaccharide are being prepared at this particular pH to confirm the above results, with a view to adopt this modified method for routine preparation of the polysaccharide.

Polyvaccine against cholera and typhoid group of infections (An ICMR Enquiry under Shrivastav, J. B.)—Polysaccharide fractions obtained from *V. cholerae* Inabe 569B by co-precipitation method have been pooled from 6 batches (about 2.0 gm) to have sufficient amount for carrying out various tests. The pooled sample is being tested by active mouse protection test along with a batch of cholera vaccine to correlate the efficacy of the extracted fraction with that of the cellular vaccine. The preliminary work shows that the effective immunising dose is as low as 0.001 mg. Toxicity of the preparation was also tested and a minimum lethal dose of 1.75 mg. was determined when injected intraperitoneally in mice. For carrying out passive protection test the antiserum against polysaccharide preparation is being raised in rabbits.

3. *Biological Standardisation and Quality Control Section and National Salmonella and Escherichia Centre*

(1) 0-1 *Phage lysis in Salmonella species in India (Sharma, V. K. and Agarwal, S. C.)*—Susceptibility to phage lysis has been frequently used for the determination of salmonella species. Felix and Callow discovered that salmonella paratyphi B, 0:1 phage lysed salmonella species belonging to somatic groups other than group B. Cherry and co-workers in 1951 advocated the use of 0-1 phage in the identification of Genus Salmonella. Salmonella infections are very common in our country and the present investigation was undertaken to explore the use of phage 0:1 lysis in the detection of salmonella serotypes commonly occurring in India. The specificity of phage 0:1 phage lysis for salmonella strains was determined by testing bacteria belonging to other genera of Family Enterobacteriaceae. All the bacteria examined in this study had been obtained during the last four years at this Centre for identification and serotyping, from different hospitals, Public Health Laboratories and Veterinary colleges in the different parts of the country.

Altogether 794 salmonella strains were examined for sensitivity to salmonella paratyphi B, 0-1 phage. These strains belonged to 26 salmonella serotypes prevalent in India. 13.6 per cent of these Salmonella typhi strains were resistant to phage lysis. The importance of this observation is enhanced when it is realised that about 85 per cent of the salmonella infections in this country are due to salmonella typhi. Consequently if phage 0-1 is used for detection of Salmonella species in India about 13.5 per cent Salmonella typhi strains will not be lysed and therefore missed. Other serotypes which contain resistant strains are Salmonella anatum, Salmonella paratyphi A, Salmonella typhi-murium, Salmonella chester and Salmonella metopeni.

Serotypes susceptible to lysis are salm. aberdeen (2), Salm. bareilly (4), Salm. bovis-morbificans (5), Salm. brunei (4), Salm. champaign (2), Salm. cholera suis (1), Salm. colombo (1), Salm. dublin (1), Salm. enteritidis (7), Salm. newport (2), Salm. paratyphi C (3), Salm. poona (12), Salm. richmond (13), Salm. salford (1), Salm. sandiego (3), Salm. stanley (2), Salm. weltevreden (2), Salm. worthington (2), Salm. virchow (1) and Salm. pomona (1)

The specificity of 0-1 phage was tested on organisms belonging to other genera of the Family Enterobacteriaceae. These organisms were shigella (26), escherichia coli (106), Klebseilla (28), Aerobacter (40), and Proteus (17). Lysis was observed only in one strain each of Escherichia coli and Aerobacter. Hence it appears that 0-1 phage lysisc is highly specific for Genus Salmonella and it can be used for the detection of Salmonella genus.

It has been concluded from this study that (i) 0-1 phage lysis should not be entirely depended upon for detection of salmonella serotypes, (ii) Doubtful strains of salmonella giving no lysis with phage 0-1 should be tested with salmonella Vi serum and polyvalent 'O' serum, (iii) A strain giving a positive lytic zone with phage 0-1 is very likely to be a salmonella and it should be confirmed with other biochemical and serological tests, and (iv) 0-1 phage should be very useful for the detection of new salmonella serotypes.

(2) *Study of the Bacterial Flora in Diarrhoeas in children at Patiala (Agarwal, S. C. and Ramakumar, L.)*—The bacterial flora in 49 children under 5 years of age have been examined to isolate organisms responsible for the clinical symptoms. Escherichia coli have been isolated in 16 patients. In 8 cases Escherichia coli is the only organisms isolated while in the other 8 cases it is associated with other organisms, Streptococcus faecalis (1); Alkaligenes faecails (1); staphylococcus albus (2); Pseudomonas aeruginosa (3) and Providence (1). Only 4 strains of Escherichia coli could be serotyped with the 15 Escherichia coli antisera prepared against 15 enteropathogenic coli. The serotypes found were 020 (2); 0119 (1) and 0125 (1). The other organisms isolated from these cases are Staphylococcus (9), Streptococcus faecalis (12); Arizona (1); Aerobacter aerogenes (1); Aerobacter cloacae (1); Proteus (4) of which 1 is Proteus mirabilis; Providence (1); Alkaligenes faecalis (5); Pseudomonas aeruginosa (9) and Gram positive aerobic spore bearers (5).

This study shows that amongst the Enterobacteriaceae E. coli were the most frequently isolated organisms in the diarrhoeas of children. Strains belonging to other genera like aerobacter; Aerogenes, proteus and Providence groups were infrequent. Salmonella and Shigella infections were almost completely absent. Among the organisms belonging to Non-enterobacteriaceae groups, Streptococcus faecalis was the most frequently isolated organism and Pseudomonas aeruginosa came next in frequency.

4. *Influenza Centre*

Attempts to isolate the virus from cases clinically resembling Influenza are being carried out. Arrangements have been made with the local hospitals to obtain throat washings of such cases. The throat washings are cultured after suitable treatment in embryonated eggs. No isolation of influenza virus has been made so far. Attempts at isolation of virus is being continued.

High titre sera against the different strains and types of influenza virus have been prepared. The work of preparing these sera has been

hampered by the lack of sufficient number of suitable laboratory animals. In the absence of rabbits attempts were made to raise the sera in guinea pigs.

It was found that guinea pigs do not react well to immunisation with influenza viruses when it is carried out with 3 intraperitoneal injections of the virus suspension as is done in rabbits. It was, however, seen that guinea pigs also responded well if the intraperitoneal injections of virus were given 4 to 6 weeks after the animals were subjected to intranasal instillation of allantoic fluid containing high concentration of the homologous stain of virus. Sera prepared in this way have been found to be satisfactory after suitable treatment for destroying non-specific inhibitors for identification of influenza viruses by haemagglutination-inhibition test.

Samples from the bloods received for preparation of Dry Plasma have been collected and stored after separation of the cells. Haemagglutination-inhibition tests are being carried out on these specimen to find out the pattern of antibody contents in this group against influenza and sendai viruses.

This work is in progress.

5. *Serum Concentration Section*

1. *ICMR Cholera Endotoxin Enquiry under Dr. E. K. Narayanan*—The work was done on the following aspects—

(i) Production of diarrhoea in young rabbits by oral feeding of *V. cholerae* endotoxic preparations.

(ii) Permeability enhancement of excised intestines of guinea-pigs exposed to the action of chloroform lysate of *V. cholerae* cells grown anaerobically.

(iii) Relationship, if any, between De's (1959) enterotoxin and extra cellular enzymes of *V. cholerae.*

(iv) Identification of the source and nature of proteins and their degradation products in cholera stools.

1. Dutta and co-workers (1959) supported Burrow's endotoxic theory on the basis of their results of production of diarrhoea in young rabbits (8—12 days old) fed orally with the "endotoxin" of *V. cholerae* prepared according to Callut's (1954) method. As reported last year no diarrhoea could be produced in young rabbits (8—10 days old) fed with Gallut's "endotoxic" preparations from an Inaba 569B strain of this Institute in 12 experiments. It was suggested by the Cholera Advisory Committee that experiments should be repeated using Dutta's rabbit passaged *V. cholerae* Inaba 569B strain. Steps are being taken to repeat the work with this strain

2. As the chloroform lysate preparations from anaerobically grown *V. cholerae* cells gave positive permeability changes in guinea-pigs excised intestinal pieces in four experiments reported last year, it was considered of interest to verify this on a larger number of experiments. Twenty one experiments in all were done and the average percentage variation in

permeability of experimental membranes in contact with lysate suspension as compared to controls in Ringer-Locke solution was —6.3% (Table I).

TABLE I

Serial No. of Expt.	Volume of fluid flowing in 2 hours		Percentage difference in flow rate of experimental w.r.t. control membrane	Average variation %
	Control Membrane (In Ringer-Locke solution)	Experimental membrane in lysate solution		
1	0·40 c.c.	0·41 c.c.	+2·5	
2	0·49 c.c.	0·81 c.c.	+77·5	
3	0·34 c.c.	0·69 c.c.	+102·9	
4	0·34 c.c.	0·47 c.c.	+38·5	
5	0·56 c.c.	0·48 c.c.	—14·3	
6	0·92 c.c.	0·67 c.c.	—27·1	
7	0·56 c.c.	0·62 c.c.	+10·7	
8	0·62 c.c.	0·83 c.c.	+33 8	
9	0·77 c.c.	0·42 c.c.	—45·2	
10	0·39 c.c.	0 27 c.c.	—30·8	
11	0·23 c.c.	0·33 c.c.	+14·3	
12	0·43 c.c.	0·36 c.c.	—16·2	
13	0·34 c.c.	0·26 c.c.	—23·5	
14	0·59 c.c.	0·36 c.c.	—39·0	
15	0·41 c.c.	0·26 c.c.	—36·5	
16	0·60 c.c.	0·48 c.c.	—20·0	
17	0·53 c.c.	0·40 c.c.	—24·5	
18	1·06 c.c.	0·86 c.c.	—18·8	
19	1·54 c.c.	0·55 c.c.	—64·2	
20	0·61 c.c.	0·38 c.c.	—37·7	
21	0·41 c.c.	0·35 c.c.	—14·6	Average—6·3%

3. —De (1959) and De *et al* (1960) reported that bacteria-free filtrates designated as "enterotoxin" obtained from *V. cholerae* grown in 5% Bacto-peptone gave positive loops in rabbit intestine. Since the "enterotoxin" is an extracellular material from *V. cholerae,* it was considered necessary to establish the relationship if any of such a product with the enzymes of *V. cholerae* already referred to in the past annual reports. These enzymes were shown to have given positive permeability changes in isolated intestinal pieces of guinea-pig's ileum. Enterotoxic filtrates were prepared according to De's (1959) method from *V. cholerae* Inaba 569B and the effect of such filtrates on the permeability of isolated guinea-pig's intestines was studied. The results of 11 experiments done are given in Table II.

Effect of *V. cholerae* "Enterotoxin" on the permeability of mesentery-free intestinal pieces of guinea-pigs. Pairs of pieces which showed equal permeability to R.L. Solution in a preliminary period of $\frac{1}{2}$ hour were compared. Enterotoxin filtrate was prepared according to De's (1959), *De et al's* (1960) method using 569B Inaba Strain.

(The filtrate was introduced inside the pipette, thus contact being with the lumen of the intestine).

Serial No. of Expt.	Volume of fluid flowing in 2 hours		Percentage difference in flow rate of experimental w.r.t. control membrane	Average variation %
	Control Membrane (In Ringer-Locke solution)	Experimental Membrane (Acted upon by "Enterotoxin")		
1	0·45 c.c.	1·10 c.c.	+144·4	
2	0·78 c.c.	1·17 c.c.	+50·0	
3	1·02 c.c.	1·39 c.c.	+36·2	
4	1·02 c.c.	1·46 c.c.	—54·0	
5	0·85 c.c.	1·21 c.c.	+42·3	
6	0·74 c.c.	1·06 c.c.	+44·4	
7	0·55 c.c.	0·80 c.c.	+45·4	
8	0·55 c.c.	0·67 c.c.	+21·8	
9	0·34 c.c.	1·02 c.c.	+200·2	
10	0·34 c.c.	0·28 c.c.	—18·0	
11	0·34 c.c.	0·48 c.c.	+41·0	+50·2% for 11 expts.

The results were unmistakably positive, the average percentages variation of experimental membranes w.r.t. controls being +50.2%.

One of these filtrates (No. 1, Table II) was then tested in *in situ* loops of two rabbits as per De and Chatterjee's (1953) technique. The experimental loop was positive only in one rabbit, while the control loops in both the rabbits were negative. There was shortage of rabbits in the Institute, and so it became necessary to experiment on guinea-pigs as per Veerraghavan (1960).

One of the filtrates prepared from *V. cholerae* Inaba 569B, gave negative results in isolated pieces and also in *in situ* loops of guinea-pig's intestine. But it was later found that the organism had turned rough. The filtrates prepared from some NAG strains taken from an old stock did not give positive permeability changes. However, work is on hand in the search of good enzyme producing NAG's.

5. In connection with the question whether intestinal tissue damage exists in cholera, one way of shedding light on the problem is to ascertain the nature and possible source of the protein in cholera stool which according to GHOSH and CHAKARBORTY (1940) exists in it to about 0.95%. For identifying the protein in Cholera stools high titre precipitin serum has been raised in rabbits against normal human serum. Techniques have been developed for obtaining seitz filtrates of both normal and cholera human stools under field conditions.

The filtrability of an emulsion of normal human stool has been found to be better when the stool corresponds to a fat-free diet. In two cases of normal stool from a person with high protein diet, the stool filtrate has been found to be free from protein. Serum added to such stool emulsion

have been recovered in about 50% of the added concentration. The technique of obtaining sterile filterates of human stool has a valuable applicability in the study of copro antibodies after cholera immunisations. It is proposed initially to make stool studies in cholera patients should an opportunity arise in or around Delhi.

6. *Triple Vaccine Section*

The Triple Vaccine Section which was started in the middle of 1961 is charged with the development of Purified Adsorbed Diphtheria, Tetanus and Pertussis Vaccines, as single, double and triple vaccines.

The work reported here therefore covers various aspects of production and development of these vaccines. It is true that standard production methods as used in laboratories abroad are available in the literature and it should be possible for a new production unit to develop these vaccines in a short time. However, most of the high grade chemicals and essential constituents of culture media are not available to us, thus necessitating research for finding indigenously available substitutes.

(1) *Selection of a suitable culture medium for preparing diphtheria toxin (Jasbir Kaur and Suri, J. C.)*—Two types of culture media were selected for trial, i.e., meat digest medium and medium prepared from locally available casein. A small amount of bacto casamino acids (Difco) became available and was used as a control.

Trypticdigest of meat. (Pope and Lingood, 1939)—This medium gave toxin production regularly but the purity of the crude toxoid was in the range of 260—750 Lf/mg P.N. With a view to improve the purity of the final product, an attempt was made to decrease the protein nitrogen initially present in the medium by increasing the number of additions of trypsin from 10 to 12 and also increasing the time of digestion. No significant change in Lf/ml or increase in purity was noticed, because the protein nitrogen/ml in the medium could not be reduced by this modification. Attempts are being continued to increase the purity of the crude toxoid by employing papain instead of trypsin for digestion of meat.

Casein hydrolysate digested locally—Although the purity of the crude toxoid reached in this case, varies from 700—2,000 Lf/mg P.N. this medium did not improve satisfactory as toxin yield was not regular. While some batches gave Lf as high as 80 Lf/ml other batches prepared in similar manner did not give any toxin. The type and source of casein used for the preparation of the medium by itself has profound effect on the yield of toxin. Since commercial suppliers are not able to give the same product each time, it is difficult to standardise the method. Trials are being made to find out some yard-stick by which the quality of the casein and its digest regarding toxin production can be defined. Biochemical analysis of different components of the digest is being done at each stage to find out, if possible, a specific factor which might be responsible for liberating the toxin from the bacterium. Keeping in view these objects, casein has been digested in the following three ways—

(i) *Ten hours hydrolysis with* 8 *N.H cl. (Holt's method)*—In this method the excess of HCl has to be removed by vacuum distillation. The process is tedious and time consuming and hence not suitable for large scale production. Moreover, by this method toxin production though good is not regular.

(ii) 72 *hours hydrolysis with a mixture of HCl and* H_2SO_4—In this method the distillation of HCl is avoided and H_2SO_4 can be easily removed by barium carbonate. Some batches digested in this way have given results.

(iii) *Tryptic digest of casein*—This method is much simpler than the two mentioned above, but so far, it has not given any toxin production. Details of the results of these trials are given in Table I.

It was interesting to note that with all these digests although the bacterial growth was good toxin was present only in a few batches.

TABLE I

Trials for Casein Hydrolysis (Locally Digested)

Batch No.	Digested with	Dilution	Accessories added	Growth	Lf/ml
CH 32/62	(i) HCl	10%	0 2% growth factor	Good	10
CH 32/62	(i) HCl	15%	Do.	Good	Nil
CH 32/62	(i) HCl	10%	0·5% growth factor	Good	Nil
CH 32/62	(i) HCl	7%	Do.	Good	Nil
CH 32/62	(i) HCl	10%	0·2% growth factor + 0·37% 0·37 % Sodium Lactate + 0·25% glycine	Good	40
CH 32/62	(i) HCl	10%	0·2 % growth factor + 0·37% lactic acid + 0 25% glycine	Good	70
CH 31/62	(i) HCl	10%	0·2% growth factor	Good	Nil
CH 31/62	(i) HCl	10%	0·2% growth factor + yeast extract	Good	Nil
CH 31/62	(i) HCl	7%	0·2% growth factor + yeast extract	Good	Nil
C.H.I.	(iii) Trypsin	Concentrated	0·2% growth factor	Good	Nil
C.H.I.	(iii) Trypsin	50%	0·2% growth factor	Good	Nil

Since each type of digestion of casein might give different combination of amino acids in the hydrolysate, trials were made by mixing the hydrolysate from different types of digestion and by mixing batches of hydrolysate which gave good toxin with those that did not. This was done with a view to make up for any deficiency of the nutrients in the batches of hydrolysate which did not give good toxin. The results of these trials are given in Table II.

TABLE II

Trials for Casein Hydrolysate (different types of digestions)

Batch No.	Digested with	Dilution	Accessories	Growth	Lf/ml
CH 33/62	(i) HCl	8%	0·2% growth factor	Good	60
CH 1	(iii) Trypsin	10%	Do.	Good	Nil
CH 33/62	(i) HCl	7%	equal volume Do.	Good	Nil
CH 1	(iii) HCl	10%	mixed		

TABLE II—*contd.*

CH 32/62	(i)	HCl	7%	mixed	Good	70
	(iii)	Trypsin	10%	Do.	Good	Nil
CH 32/62	(i)	HCl	7% equal volume	Do.	Good	Nil
CH 1	(iii)	Trypsin	10% mixed			
CH 31/62	(i)	HCl	10%	Do.	Good	Nil
CH 32/62	(i)	HCl	10%	Do.	Good	70
CH 31/62	(i)	HCl	10% equal volume	Do.	Good	Nil
CH 32/62			10% mixed			
CH 31/62	(i)	HCl	10%	Do.	Good	Nil
CH 33/62	(i)	HCl	8%	Do.	Good	60
CH 31/62	(i)	HCl	10% equal volume	Do.	Good	Nil
CH 33/62	(i)	HCl	10% mixed			

It was found that in most cases, addition of glycine and lactic acid favoured the yield of toxin.

Efforts are being made to standardize the conditions of digestion, decolourisation and deferration of the casein digest medium.

(2) *Selection of a suitable culture medium for preparing tetanus toxin* (*Bytchenko and Suri, J. C.*)—(i) The production of fluid tetanus toxoid prepared from meat digest medium was discontinued during the year and instead Mueller's medium prepared from locally available casein was adopted. Some batches of casein digest yielded a good toxin while others did not. Results of the first 17 batches of toxin prepared on this medium is shown below:—

Tetanus toxin yield in terms of M.L.D./ml and Lf/ml of 17 batches prepared from tryptic digest of casein (locally prepared)

M.L.D./ml	Number of batches having the Lf/ml value of			
	0—10	>10—20	>20—40	>40
<10,000	1	1	2	..
10,000	1	2	..	..
100,000	..	1	..	..
1,000,000	4	3	1	1

12,600 c.c. of the Formolinised Tetanus Toxoid (F.T.) were supplied to the Stock Room of the Institute for issue.

On receipt of the N Z C [illegible] the U.N.I.C.E.F., it became possible to run a few [illegible] toxin [illegible] from N Z C [illegible]. The results of the [illegible]

M L D [illegible]

MLD/ml	[illegible]				
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]					2
[illegible]	[illegible]	[illegible]	[illegible]		.
[illegible]				1	2
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2
[illegible]					1

(ii) The other media which were [illegible]

[illegible] and [illegible]

Culture medium	*(Ingredients)*
1. Glasman's medium	(i) Meat hydrolysate.
	(ii) Meat infusion.
	(iii) Glucose.
	(iv) Reduced iron.
2. Casein fish meal.	(i) Casein hydrolysate.
	(ii) Fish meal.
	(iii) Maize extract.
	(iv) Yeast.
	(v) Glucose.
	(vi) Reduced iron.
3. Vegetable casein medium.	(i) Casein hydrolysate.
	(ii) Yeast extract.
	(iii) Wheat husk extract.
	(iv) Glucose.
	(v) Red. iron.
4. Casein meat infusion.	(i) Casein hydrolysate.
	(ii) Meat infusion.
	(iii) Yeast extract.
	(iv) Maize extract.
	(v) Glucose.
	(vi) Reduced iron.

The toxin produced on these media was measured by flocculation units (Lf/ml) and by a double diffusion test in agar plates. Later it became apparent that by themselves these two methods of measuring tetanus toxin are very erroneous, since mutant used for inoculating these culture medium was demonstrated by in-vivo test to be atoxigenic. Because of this accidental error, proper evaluation of these culture media could not be

made. This investigation will be taken up again when the supply of mice (for in-vivo test) has improved.

(3) *Examination of soil samples (Bytchenko and Pant, R)*—50 soil samples were collected from different areas in Kasauli and cultured for the presence of Cl. Tetanii. Two samples were found positive.

(4) *Concentration and purification of the Crude Diphtheria and Tetanus Toxoids (Suri, J. C. and Kaur, J.)—Ultrafiltration*—Since kidney filters were not available we tried L3 candles and Berkfeld filter candles (House-hold type) and found them suitable for this purpose. The only draw-back with these is that as the surface area is small the rate of filtration is slow and therefore for large scale production multiple units have to be put up. *Parlodion* which is the standard substance for coating these candles for purposes of ultrafiltration of the toxoids was not available to us in the beginning. We tried other substitutes available in the country. One of these substitutes is Pyroxlin (B.D.H.).

Pyroxlin (B.D.H.)—Different concentrations, i.e., 4%, 5%, 6%, 7% and 8% have been tried to ultrafilter the toxoid. It was found that 5—7% concentration gave the best results.

As pyroxlin is also an imported product and is costly, trials are being made to use gun-powder which is manufactured by the Ordinance Factories in India.

Field work—Schick Test Surveys (Suri, J. C.)—An attempt was made to do a schick test survey among pre-school age children in Chandigarh. The test was given to 300—400 infants and children, at Sector 19 and 22 Health Centre. However, only 135 children turned up on the day the test was to be read. Result of these is tabulated below: —

Schick test survey—19 and 22 Sector, Chandigarh, March, 1962

Age	Number inspected	Number negative	Number positive	Percentage positive
				%
0—6 months	7	6	1	14·2
—1 year	22	2	20	90·9
—2 years	34	5	29	85·3
—3 years	33	10	23	69 7
—4 years	15	8	7	46·7
—5 years	20	12	8	40 0
—6 years	4	2	2	50·0
Total ..	135	45	90	66·7

[illegible] of the [illegible] Test [illegible] School Children [illegible] are given in the Table [illegible]

Results of [illegible]
196[illegible]

Age	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]		
[illegible]				
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Above [illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Total	[illegible]	[illegible]	[illegible]	[illegible]

Tetanus Neonatorum (Sen, J. G.) — A field project on the [illegible] of immunization of pregnant mothers with [illegible] was started in 1961 at the Cantonment General Hospital, Kasauli [illegible] centres at Chandigarh. Cord blood samples are collected at the time of deliveries to assess the efficacy of the immunization schedule.

During the year 1962, 121 samples were collected. Out of which 75 have been tested for Tetanus Antitoxin Titre. The results of these trials will be reported after sufficient samples have been tested.

During the year 1961 and 1962, Fluid Tetanus Toxoid was used for immunization. It is now planned to replace it by Purified Adsorbed Tetanus Toxoid and study the improvement in the toxin titres in Cord Blood.

Field trials on Purified Adsorbed Diphtheria Toxoid (Sen, J. G.) — Since one of the objectives before the Triple Vaccine Section is to evolve a very potent diphtheria toxoid, so that the number of injections can be reduced it has been decided to prepare experimental batches of vaccine with varying quantities of the adjuvant (Aluminium phosphate) and diphtheria toxoid. To start with, four different compositions of purified adsorbed diphtheria toxoid have been selected for trial, namely —

(i) Diphtheria toxoid Aluminium phosphate ... 30 Lf/ml 3 mg/ml

(ii) Diphtheria toxoid Aluminium phosphate ... 50 Lf/ml 3 mg/ml

(iii) Diphtheria toxoid Aluminium phosphate ... 50 Lf/ml 5 mg/ml

(iv) Diphtheria toxoid Aluminium phosphate ... 30 Lf/ml 5 mg/ml

Organisation of these field trials is being finalised in consultation with the Directorate of Health Services, Punjab. It is expected that these trials will start at Kasauli, Chandigarh, Ludhiana and Samrala early in 1963.

Duration of passive immunity after administration of human tetanus antitoxin (Suri, J. C.)—This project which commenced in 1961 involved injecting into volunteers—Indian and Europeans—a dose of commercially available Tetanus Immune Globulin and then collection of blood samples at frequent intervals. These samples were then assayed in-vivo for Tetanus Antitoxin titre. This study was done in collaboration with the Department of Bacteriology, University of Melbourne, Australia. The results of this investigation completed in 1962 have been published in B.M.J. (1962), Vol. *i*, p. 79—81.

An unexpected finding has been that the Immune Globulin was more rapidly excreted in Indian volunteers as compared to the European volunteers. A more detailed investigation to confirm these findings has been planned with the financial aid from the Indian Council of Medical Research.

7. *Virus Section*

Virus neutralising antibody level in Serum of Patients receiving antirabic treatment—Twenty six samples of blood from patients undergoing different schedules of treatment have been collected during the course of year.

Out of these 8 samples are from patients who received Anti-Rabies Serum followed by 14 daily injections and one booster dose a week after the 14th injection of Anti rabies vaccine. It has not been possible to titrate all the specimens of sera due to non-availability of experimental animals.

In the few case tested the pattern of antibody level observed has been the same as reported in the previous years. After the administration of Serum the antibody levels on the 15th day are found to be low in a small proportion of cases, while the sera collected after the administration of the booster dose showed high levels of neutralizing antibody.

2. *Avianised live street virus vaccine*—The strain of street virus which has lost its pathogenicity to dogs, rabbits and guinea pigs due to avianisation has now been employed to prepare an avianised vaccine for animals. It is a 50 per cent suspension of infected chick embryo and has been successfully freeze-dried. It is awaiting trials.

3. *Study of Antibody level due to Chick Embryo vaccine*—Experiments have been carried out to find out if the immunity after a course of 4 injections of Antirabic Vaccine prepared from brain tissue followed by 10 injections of inactivated avianised vaccine is as good as that obtained following 14 injections of brain vaccine.

Avianised vaccine was prepared from a fixed virus strain adapted to eggs in its 26th egg passage level. The titre of the strain in mice was 10^{-5}. A 10 per cent emulsion of the embryos in buffered saline with 1 per cent

carbolic acid was incubated at 37°C for 24 hours before dilution with an equal volume of buffer to make it a 5 per cent emulsion. 1/10 dilution the 5 per cent emulsion was injected intracerebrally in mice for testing th inactivation of the virus. The vaccine was not found to contain acti virus particles.

The brain vaccine used in the experiment was the routine 5 per cen antirabic vaccine prepared from a pool of 30 infected sheep brains.

Three immunization schedules as given under were tried in t groups of monkeys, each group consisting of two monkeys—

Group I—5 injections of 1 c.c. of brain vaccine followed by 9 i jections of 1 c.c. each of chick-embryo vaccine.

Group II—14 injections of 1 c.c. each of 5 per cent chick vaccine.

Group III—14 injections of 1 c.c. each of 5 per cent brain vaccine.

Each group of animals were bled on the 10th, 15th and 30th day fr the date of commencement of immunization. Neutralization test with in activated sera were put up in mice against a virus titre of 50 LD_{50} in th final dilution.

The following dilutions of sera were tested—

Serum collected on the 10th day—1:4, 1:8 and 1:16.

Serum collected on the 15th day—1:8, 1:16 and 1:32.

Serum collected on the 30th day—1:16, 1:32 and 1:64.

All the three samples of sera from animals under group I and III pro tected all animals in all the dilutions while sera from group II animal (receiving only chick vaccine) were not found to be as satisfactory as th other two groups.

Experiment on the same pattern using a large number of guinea-p per group is now being carried out.

It may be expected that a course of 4 or 5 injections of brain vacci followed by inactivated avianised vaccine would give a high degree of immunity and at the same time completely eliminate the possible risk of paralytic accidents. However, the incidence of local and general reactions due to chick embryo vaccine need to be studied in greater detail.

4. *Tissue culture of rabies virus*—Work on tissue culture adaptation of the avianised fixed virus strain is still in progress. The titre of the virus so far obtained in the culture fluid is still too low to encourage preparation of vaccine from Tissue Culture Fluid.

5. *Manufacture of Yellow fever vaccine*—Manufacture of yellow fever vaccine has been started in this Institute and some batches have been sent to three laboratories designated by W.H.O. for testing. The report from W.H.O. is awaited before the vaccine can be released for vaccination.

Seed virus—The seed virus used for the manufacture of vaccine has been supplied by "Institunt Voor Tropiche Hygiene En Geographische Pathologie". It is at the 249th sub passage level of 17D strain of yellow fever virus and has been approved by the W.H.O.

Procedure—0.06 c.c. of 1:25 dilution of Seed Virus in sterile double distilled water is inoculated in amniotic Sac of 8 day old healthy embryonated eggs. The inoculated eggs are further incubated for three days and the living eggs at the end of incubation are harvested and a 66 per cent emulsion of the embryos is made in sterile double distilled water. This emulsion is centrifuged at 2000 r.p.m. for 30 minutes and the supernatant so collected is ampouled and lyophyised in a lyophylisation Unit designed at this Institute.

The dose per ampoule is calculated on the basis of virus content in that particular batch giving 5,000 to 10,000 50 dose mouse unit per human

Each dried batch is tested for toxity in Guinea-pigs and also for moisture content, sterility and total protein Nitrogen in conformity with the rules framed by the World Health Organisation.

IV. ROUTINE WORK CARRIED OUT DURING 1962

(I) *Bacterial Vaccines Section*—(i) The quantities of bacterial vaccines manufactured and issued during the year 1962 are given below—

Products	Quantity manufactured (concentrated vaccine)	Quantity processed	Quantity issued
Anti-cholera vaccine	37,74,000 c.c.	31,07,900 c.c.	27,40,686 c.c.
T.A.B. vaccine	15,21,000 c.c.	14,04,200 c.c.	12,18,314½ c.c.
Curative vaccines	..	2,839 doses	2,839 doses

Prophylactic cholera vaccine—This is an agar-grown product killed and preserved with carbolic acid and prepared from equal parts of Inaba and Ogawa sub-type, giving a total strength of 8,000 million organisms per c.c. of vaccine.

Issues during the year 1962 amounted to 27,40,686 c.c. of this 26,53,865 c.c. were issued to civil indentors and the balance to the Defence services.

Prophylactic Mixed T.A.B. vaccine—T.A.B. vaccine is an agar-grown heat killed phenol-preserved vaccine containing 1,000 million Bact. typhosus (500 million each of Bact. typhosus Rawling, and of a locally isolated Vi strain) and 500 million each Bact. paratyphosus A and B isolated in India.

Total issues of this vaccine in 1962 were 12,18,314½ c.c. Of this, 9,62,356 c.c. were issued to Defence services and 2,55,958½ c.c. to civil indentors.

Curative stock and autogenous vaccines—2,839 doses of these vaccines were supplied during the year 1962. The chief issues were mixed influenzal and catarrhal vaccine, special autogenous vaccines and specially diluted T.A.B. vaccine for protein shock therapy.

The details are given below—

	Doses
(A) Autogenous Vaccines	169
(B) Stock vaccines—	
Mixed influenzal and catarrhal vaccine	1,695
Mixed staphylococcus and streptococcus	60
Mixed acne and staphylococcus vaccine	12
B. coli vaccine	40
Gonococcus vaccine	174
T.A.B. vaccine for protein shock therapy	633
Malta fever vaccine	48
Mixed staphylococcus and gonococcus vaccine	8
Total ..	2,839

(ii) *Preparation of diagnostic reagents*—Large quantities of agglutinable bacterial suspensions, high titre sera, wassermann antigen and anti sheep haemolytic serum were prepared and issued to various Governmen hospitals, laboratories, and private laboratories in different parts of the country.

Agglutinable suspensions—A total of 101,620 c.c. of various suspensions was issued during the year 1962. The details are given below—

Name of suspensions	Quantity issued
	c.c.
1. B. typhi 'H'	29,790
2. B. typhi 'O'	15,955
3. B. paratyphi A 'H'	20,365
4. B. paratyphi A 'O'	10,745
5. B. paratyphi B 'H'	16,540
6. B. paratyphi B 'O'	6,840
7. B. paratyphi C 'H'	1,185
8. B. paratyphi C 'O'	970
9. B. melitensis	3,310
10. B. coli communis	210
11. B. coli communior	10
12. B. proteus OX19	1,850
13. B. proteus OXK	1,260
14. B. proteus OX2	1,370
15. Sh. dysentery Shiga	10
16. Sh. dysentery Flexner	10
17. B. paratyphi 'B'	200
Total ..	101,620

High Titre Sera—A total of 1,106 c.c. of various High Titre Sera was issued during the year 1962. The details are given below—

Name of H.T. Sera	Quantity issued
	c.c.
1. B. typhi 'H'	148
2. B. typhi 'O'	111
3. B. paratyphi A 'H'	125
4. B. paratyphi A 'O'	14
5. B. paratyphi B 'H'	111
6. B. paratyphi B 'O'	52
7. B. paratyphi C 'H'	39
8. B. paratyphi C 'O'	37
9. Br. Melitensis	26
10. B. typhi VI	10
11. B. proteus OX19	15
12. B. proteus OXK	15
13. B. proteus OX2	15
14. Sh. dysentery Shiga	81
15. Sh. dysentery Flexner	87
16. V. cholerae (non-differential)	88
17. V. cholerae (mono-specific) Inaba	19
18. V. cholerae (mono-specific) Ogawa	90
19. V. cholerae Rough 'O'	18
20. Salm. OA	1
21. Salm. OB	1
22. Salm. OC	1
23. Salm. OD	1
24. Salm. OE	1
Total ..	1,106

NOTE—Items No. 16 to 24 were supplied by National Salmonella and Escherichia Centre, P.I., Kasauli.

Anti-sheep haemolytic serum—During the year 1962, 203 c.c. of this serum was issued.

Wasserman Reagents, issued during the year 1962—

	c.c.
Antigen cholesternized	300
Antigen Goat's Heart	140
Cholesterin 1% solution	65

(iii) *Distilled water production*—During the year 1962, 25,741 litres of distilled water were produced for dilution of Antirabic vaccine, manufacture of normal saline, dilution of bracterial vaccines and other laboratory use. In addition, 12,050 tubes of 5 c.c. each of Distilled water for injection (I.P) were manufactured during this year for issue to outside indentors.

(iv) *Other units*—(a) Besides manufacture of biological products, the Bacterial Vaccines Section also runs the Ampoule Blowing; Ampoule Filling and Sealing and Labelling Sections of the Institute.

Agency requesting test	Products	No. of samples tested	Results	
			Standard quality	Sub-standard quality
Inspector of Drugs, West Bengal, Calcutta.	Triple antigen ..	1 (Tested for tetanus toxoid diphtheria toxoid).	1	..
Novo Pharmaceuticals Co., Ltd., Cuttack.	T.A.B. Vaccine ..	1	1	..
Divisional Inspector of Drugs Control, Patiala.	Tetanus antitoxin	1 (Potency and total solids).	1	..
Divisional Inspector of Drugs Control, Jullundur.	T.A.B. Vaccine ..	2	2	..
Inspector of Drugs, Allahabad	Penicillin ..	1	1	..
Drugs Inspector, Mangalore Division, Mangalore.	Penicillin	2	2	..
Cattle Utilization Officer, Dehra Dun.	Catguts ..	2	..	2
Total No. of samples tested ..		184	127	57

(4) *Collection of Type Culture Centre*—The Institute maintains bacterial cultures of medical interest, and these are issued to various Government and approved research and teaching institutions and to manufacturers of biological products. The strains are supplied in lyophilised state.

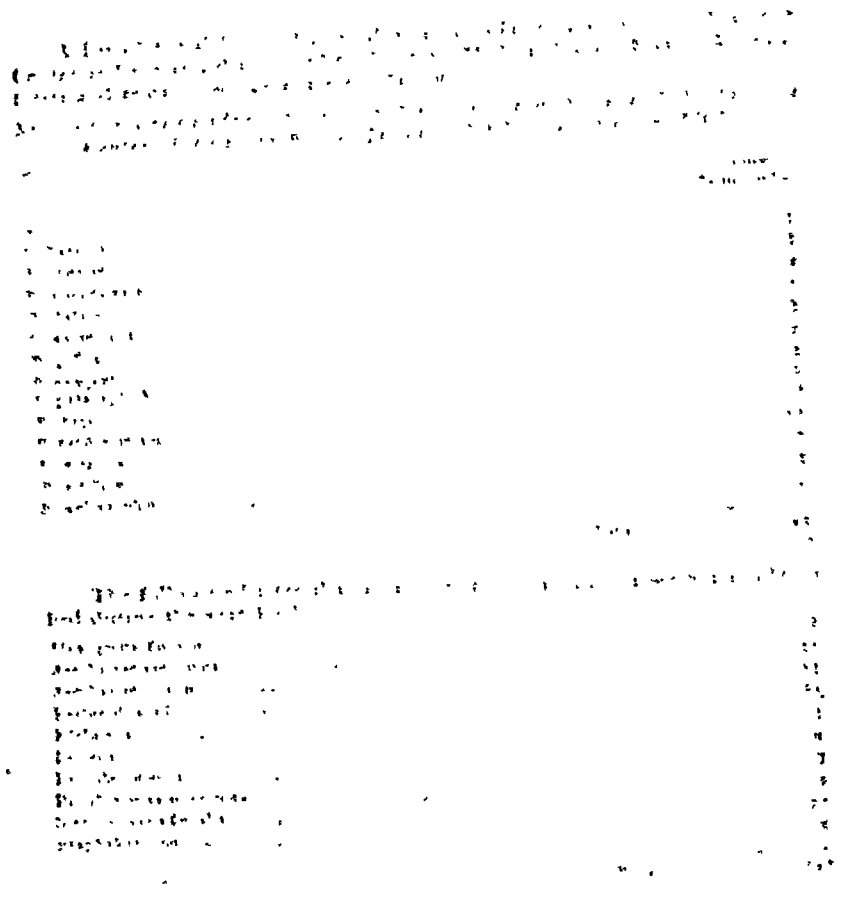
A statement showing the details of various cultures issued during the year is given below—

B. anthracis	2
A. aerogenes	2
Br. abortus	2
Br. suis	2
Br. melitensis	2
C. acne	2
C. diphtheria	9
C. xerosis	3
Chr. prodigiosum	3
Cl. tetani	2
Cl. welchii	4
H. influenzæ	4
H. pertussis	2
K. pneumoniæ	1
L. bugaricus	2
Mycobacterium (Human, Avian & Bovine) ..	8
N. meningitidis	4
N. gonorrhoeæ	4

N. catarrhalis	3
N. pharyngis	1
Proteus (OXK, OX2,019)	24
P. septica	3
P. pestis (avirulent)	1
P. pseudo-tuberculosis	2
Salmonella typhi H 901	31
Salmonella typhi 0901	10
Salmonella typhi Vi	13
Salmonell paratyphi A	23
Salmonella paratyphi B	25
Salmonella cholera suis	1
Salmonella enteritidis	3
Salmonella gallinarum	1
Salmonella typhimurium	1
Sarcina lutea	2
Shigella (flexneri, shiga, sonnei schmitz'i & boydii)	51
Streptococcus	12
Staphylococcus	7
V. cholerae (Inaba, Ogawa, Eltor & Rough)	107
V. metchnikovi	1
Total ..	380

A new catalogue of the bacterial cultures maintained at this Institute has been prepared and distributed to different laboratories in the country.

(5) *National Salmonella and Escherichia Centre*—This centre was established at the Institute in 1958 with the help of W.H.O. and Central Public Health Laboratory, Colindale, London. The main function of the Centre is to act as a Reference Centre for identification of different Salmonella and Escherichia serotypes isolated in different parts of the country. This knowledge is very valuable for analysing the incidence of Salmonella infection in various parts of the country. The centre collects all the strains obtained in this manner in a freeze dried form and maintains a catalogue of their morphological, cultural, biochemical and antigenic characters During the last three years about one thousand Salmonella strains belonging to 26 Salmonella serotypes have been indentified. Some new serotypes not reported so far in India have also been detected. An investigation was undertaken on the chloramphenicol resistance of salmonella typhi strains obtained at the centre and their results have shown that the incidence of chloramphenicol resistance has increased during the last three years. The Centre has also been preparing and distributing the somatic and flagellar salmonella group sera to different Medical and Public Health Laboratories all over the country. It has also undertaken an investigation to find out the common bacteria responsible for diarrhoeas in children and infants with special emphasis on the Escherichia coli organisms and their serotypes. This investigation has been carried out with collaboration of Department of Pediatrics, Government Medical College, Patiala.



It is understood to be intended that [illegible] the Vi phage lysate of [illegible] tried from Dr F. S. [illegible] London

Distribution of specimens [illegible] salmonella 'O' somatic and 'H' [illegible] distributed in limited quantities to the following:

1. Director, Central Drugs Laboratory, [illegible]
2. Director, Pasteur Institute of [illegible]
3. Professor of Bacteriology, Madras Medical College, Madras
4. Assistant Professor of Pathology, Medical College, Bengal

5. Head of the Division of Pathology & Bacteriology, I.V.R.I., Mukteswar.
6. Principal, S.N. Medical College, Agra.
7. Professor of Pathology, M.G.M. Medical College, Indore.
8. Head, Division of Bacteriology, N.D.R.I., Karnal.
9. Professor of Pathology, All-India Institute of Medical Sciences, New Delhi.
10. Professor of Bacteriology, Lady Hardinge Medical College, New Delhi.
11. Professor of Pathology, Veterinary College, Mathura.
12. Assistant Disease Investigation Officer, Live Stock Farms, Hissar.
13. Principal, Medical College, Trivandrum.
14. Director Medical Education and Research, Chandigarh.
15. Associate Professor of Pathology, Armed Forces Medical College, Poona.
16. Pathologist, L.T.M.G. Hospital, Bombay.
17. Principal, Christian Medical College and Hospital, Vellore.
18. Research Assistant, Veterinary College, Hissar.
19. Director, King Institute, Guindy, Madras.
20. Principal, Veterinary College, Mhow, M.P.
21. Deputy Director of Public Health and Chemical Examiner, Public Health Laboratory, Bangalore.
22. Principal, Medical College, Kurnool.

(6) *Serum Concentration Section*

The following table gives the quantities of the various biological products processed, the number of ampoules filled with the final products and the number issued to indentors—

No.	Products	Plasma processed in litres	Ampoules filled	Ampoules issued to indentors
1	Antivenom serum ..	956	13,545 of 10 ml each (liquid) 790 of 10 ml each (lyophilized).	15,271 (liquid) of 10 ml. each. 379 of 10 ml each (lyophilized).
2	Antidiphtheritic serum	129	1,756 of 10,000 units each	1,790 of 10,000 units each.
3	Antirabic serum ..	115	516 of 10 ml each .. 80 of 5 ml each.	408 of 10 ml each.
4	Antitetanus serum ..	23·5	..	.. ..
5	Normal Horse serum ..	18·86	796 of 20 ml each ..	476 of 20 ml each.
6	Tetanus Toxoid ..		150 of 50 ml each .. 510 of 10 ml each	54 of 50 ml each. 190 of 10 ml each.

(7) *Triple Vaccine Section*

During the year steps have been taken to organize the following five units—

1. Diphtheria Laboratory.
2. Tetanus Laboratory.
3. Pertussis Laboratory.
4. Vaccine Purification Laboratory.
5. Testing and Quality Control Laboratory.

Due to lack of space and personnel the Pertussis Laboratory has not yet started working. Work in all the other four units has progressively increased during the year and will expand further when more space and personnel become available. It is envisaged that in course of time, each of these component units will expand into a full-fledged department of the Triple Vaccine Section.

A great deal of developmental work on the production techniques for Purified and Adsorbed Diphtheria and Tetanus Vaccines has been carried out, in spite of the fact that work had to be suspended in several laboratories for prolonged periods to permit structural alterations in the existing building. Thus the Tetanus Laboratory really started working only in the latter half of the year.

Production of Diphtheria toxin and toxoid—Thirty eight batches of Diphtheria toxin with Lf (flocculating units) ranging from 30—60 Lf/ml were prepared during the year under report. Three different types of culture media have been employed for this purpose. Thirty batches of toxin were prepared from tryptic digest of meat made according to Pope and Linggood (1939), two batches from Mueller's medium using Bacto Casamino Acids (Difco) and the rest of the six batches from Mueller's medium but using a Casein hydrolysate prepared locally.

The superiority of Bacto-casamino Acids for making a Purified Vaccine is apparant from the table given below—

Preparation of Diphtheria toxin in 3 different media

Culture medium employed	No. of batches prepared	Lf/ml Minimum	Maximum	Average Lf/ml	Range of purity of Crude Diphtheria Toxoid Lf/mg Protein Nitrogen
Bacto Casein	2	30	40	35	680—2000
Casein Hydrolysate (digested locally)	6	25	60	40	750—1200
Tryptic digest of meat (P & L)	30	20	60	40	260—750

The toxin was then formolised and toxoided by keeping it for four weeks at 35°C. This crude toxoid was then further processed in the Vaccine Purification Laboratory.

2. *Schick test toxin and its control*—16,500 doses of Schick test toxin and an equal quantity of the control fluid (heated toxin) were prepared and issued.

3. *Diphtheria antitoxin*—In the beginning of the year, 18 horses were immunized for hyper immunization against diphtheria. At the end of the year 10 horses were being used.

294 litres of hyper immunized blood was supplied to the Serum Concentration Section of the Institute for processing.

4. *Tetanus antitoxin*—During the year 1962, 5 horses were hyper immunized for preparation of tetanus antitoxin. 30 litres of immune blood has been supplied to the Serum Concentration Section for further processing.

5. *Vaccine Purification Laboratory*—The laboratory has the following functions—

Biochemical analysis of material from the other component units of Triple Vaccine Section—A rigid analytical control is maintained at all stages of preparation of the purified diphtheria toxoid and purified tetanus toxoid. Routine analysis is conducted for the culture medium crude diphtheria toxin, crude diphtheria toxoid, ultrafiltered products and the finally purified toxoids after salting out. The analysis involves estimation of total nitrogen, protein nitrogen, amino nitrogen, chloride and Fe, free formalin etc.

5.1. *Purification of the crude toxoids*—This is done by first reducing the bulk by means of ultrafiltration and then salting out with ammonium sulphate. In this way, all the colouring materials and most of the non-specific proteins are eliminated and the resulting purified toxoid contains more than 1500 Lf/mg of protein nitrogen.

6. *Preparation of aluminium phosphate suspension*—Aluminium phosphate suspension ($AlPO_4$) is used for adsorbing the purified liquid toxoids to get the final vaccines.

Concentrated $AlPO_4$ suspension is prepared by mixing equal volumes of sodium phosphate and potash alum solutions. The ppt. formed is washed with normal saline and resuspended in fresh saline at pH 6.5. After sterilization by heat, the suspension is stored for use as and when required.

7. *Preparation of Final Vaccine*—This involves mixing with continuous stirring and under aseptic conditions AALPO, suspension and the purified and concentrated fluid toxoids in the proportions required adsorption of the toxoid on to the adjuvant is hastened by keeping the vaccine at 37°C for 48 hours.

Finally the pH of the vaccine is adjusted and the vaccine batch passed on to the Testing and Quality Control Laboratory.

During the year 30.000 doses of Purified adsorbed diphtheria vaccine and 2000 doses of Purified adsorbed tetanus vaccine were prepared.

8. *Testing and Quality Control Laboratory*—This laboratory is responsible for carrying out sterility test, potency test, toxicity test and standardization of the vaccines, at the bulk stage.

After the vaccine has been bottled, random samples are again checked for sterility and homogenicity. At this stage a further identity test and safety test is carried out, on all batches of vaccine. The latter test is a check against chance presence of tetanus spores.

Standardization and Quality Control work needs large quantities of biological standards like standard diphtheria antitoxin and tetanus antitoxin, both for in-vitro and invivo tests and standard diphtheria toxin and standard tetanus toxin etc. Since the supply of International Standards is strictly limited, working standards are prepared locally periodically for use of this section. A very strict check is kept on the preparation of these working standards.

During the year potency test on 12 batches of Purified Diphtheria Toxoid was carried out. Except for 2 batches all others gave a satisfactory potency. After increasing the concentration of the adjuvant ($AALPO_4$) in these batches from 3 mg to 5 mg these batches passed the potency test comfortably.

Two batches of purified adsorbed tetanus toxoid were tested and passed. Six batches of formalinised tetanus toxoid (F.T.) were tested, out of which only 3 batches were passed.

In addition to testing these products at the Institute several preparations were also sent to the National Institute of Public Health Utrecht, Holland, who found these products of 'excellent quality' and concluded "So I am sure you are completely on the right way and your preparations may be considered to possess the same quality as ours. Congratulations".

In addition to the standardization of the vaccine produced in the Triple Vaccine Section this laboratory also caters to the needs of the Serum Concentration Section of the Institute.

Seven samples of pooled plasma and one sample of concentrated serum were tested for the level of diphtheria antitoxins by flocculation test, and one sample of concentrated serum was tested by in-vivo method.

(8) *Virus Section*

Antirabic vaccine—The seed virus used for the manufacture of antirabic vaccine is the Pasteur Strain of fixed virus. Three sub-strains of the virus maintained at this Institute have undergone seven passage each during the year. It has been our experience that seed virus stored in 50 per cent buffered glycerine at +4°C for over a period of six months does not show any fall in its titre. For infecting sheep for the manufacture of vaccine, a 20 per cent brain emulsion suitably diluted is used. This emulsion is prepared in 50 per cent buffered glycerine by pooling Rabbit brains infected with the sub-strain. The same pool of seed virus is used for three months. Before use, the titre of the emulsion is tested in mice and identity of the virus-established by a neutralization test with a Rabies specific Serum.

The following vaccines are prepared—

(1) *Antirabic vaccine* (*human semple's*)—The vaccine is a 5 per cent sheep brain emulsion inactivated by heat and carbolic acid.

(2) *Antirabic vaccine* (*animal*)—A 5 per cent vaccine inactivated by heat and carbolic acid.

(3) *Antirabic vaccine* (*dog*)—This is a 20 per cent vaccine inactivated in the early part of the year by heat and carbolic acid and in the latter period by ultraviolet ray irradiation. This vaccine used for pre-exposure immunisation by a single injection of 5 c.c. repeated every year, is gaining more and more popularity.

The following table gives details of quantities manufactured and issued of the above mentioned vaccines during 1962—

Type of vaccine	Manufactured	Issued
	c.c.	c.c.
Antirabic vaccine (human)	44,52,529	43,36,662
Antirabic vaccine (animal)	1,31,903	1,25,785
Antirabic vaccine (dog)	43,840	42,280

(ii) *Antirabic serum*—Two horses were under immunization for the production of hyper-immune antirabic serum. The dosage schedule of immunization is the same as reported in the previous reports and has been consistently giving satisfactory titres.

(iii) *Brain examination*—A total of 165 specimens of dog brain have been received of which only 145 brains were fit for microscopic and biological examination.

Out of the 145 brains tested microscopically, 38 were found positive for rabies and 107 negative by histological examination. Of the 107, only 97 were received in glycerine and were suitable for biological test. Of these, rabies virus was isolated from 19 specimens. The rest did not show any evidence of rabies.

V. LIST OF PRODUCTS MANUFACTURED AT THE CENTRAL RESEARCH INSTITUTE, KASAULI

1. Anti-cholera vaccine.
2. Anti-rabic vaccine (human).
3. Antirabic vaccine (animal).
4. Antirabic Vaccine (dog).
5. Anti-sheep haemolytic serum.
6. Anti-tetanic serum.
7. Avianised antirabic vaccine
8. Concentrated antirabic serum.
9. Curative vaccines of different types.
10. Daboia venom solution (haemostatic).
11. Dead bacterial emulsions of different organisms.
12. Diphtheria antitoxin.
13. Distilled water for injection.
14. Freeze-dried cultures.
15. High titre sera against various organisms.
16. Media of various types for laboratory use.
17. Normal horse serum.
18. Polyvalent concentrated antivenom serum in liquid form and also in lyophilised form, effective against the venoms of Cobra, common krait, Russell's viper and Saw-scaled viper (Echis).
19. Purified diphtheria toxoid (adsorbed).
20. Reagents for laboratory investigations.
21. Schick test toxin.
22. Special autogenous vaccines
23. Stains of various types for laboratory investigations.
24. T.A.B. vaccine.
25. T.A.B. vaccine for protein shock therapy
26. Tetanus toxoid.
27. W.R. antigen.

VI. STABLES AND ANIMALS

The Institute maintains a stock of various species of animals for experimental purposes and the manufacture of its products, such as antirabic vaccine, antivenene, etc. A table showing the various animals kept during the year 1962 is given below.

(Statement showing the various species of animals kept at Central Research Institute, Kasauli)

	Serum Horses	Pack Horses and Mules	Goat	Normal sheep	Anti-rabic Sheep	Monkeys	Rabbits	G. Pigs	Rats	Albino Mice	Pigeons	Fowls	Chickens	Dog	Swiss Mice
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Opening balance at the beginning of the year 1962	58	2	1	4	50	6	203	1,094	732	3,889	36	14	..	..	..
Purchase made, transfers from Remount, Depots breeding at the Institute and other receipts	25	..	..	6	4,293	..	742	2,283	17	23,859	..	..	100	7	100
Total ..	83	2	1	10	4,343	6	1,035	3,377	749	27,748	36	14	100	7	100
Sold to out station to indentors and local laboratories, etc. and other mortalities due to bleeding and death	16	1	1	1	4,171	6	615	2,074	702	22,164	36	6	7	..	..
Total ..	16	1	1	1	4,171	6	615	2,074	702	22,164	36	6	7	..	..
Closing balance at the end of the year 1962	67	1	...	9	172	..	420	1,303	47	5,584	..	8	93	7	100

VII. WORKSHOP

The Workshop of the Institute consists of the mechanical, electrical and carpentary sections. During the year under review, the Workshop continued to give full support to the expanding activities of various sections by fabricating new equipment and by making alterations to the existing fixtures and equipment in order to make them suitable for the new type of work. Besides maintaining the essential services like supply of water, steam and gas, repair and maintenance of cold rooms, refrigerators, incubators and warm rooms, etc., a large number of animal cages—large and small—and a number of other essential simple equipment and accessories for the daily work of different sections were fabricated with the limited resources at our disposal.

VIII. LIBRARIES AND INDIAN COUNCIL OF MEDICAL RESEARCH UNITS

1. *Central Research Institute Library*—The Central Research Institute has been maintaining an excellent library. It has been subscribing to 75 scientific and medical journals worth Rs. 6,000 annually. There are 5,715 books and 6,000 bound periodicals in the stock.

The library is managed by a Library Committee consisting of three officers headed by the Director of the Institute. New books and journals are ordered at the recommendations of the committee.

The budget for the purchase of books and journals during the year was Rs. 10,000. Sixty nine medical and other technical books were added during the year under review.

2. *Indian Council of Medical Research Library*—The Indian Council of Medical Research Library, housed at the Institute, since 1913 comprises mainly of medical periodicals received in exchange with the Indian Council of Medical Research official organ "INDIAN JOURNAL OF MEDICAL RESEARCH".

During the year under review the library was on the exchange and free mailing list of 200 journals. 116 books worth Rs. 2580.00 (approximate value) on various aspects of medicine, received for review in Indian Journal of Medical Research were added to the library.

From this year the library started to circulate a monthly comprehensive medical bibliography to the staff of the Central Research Institute, Kasauli.

3. *Indian Journal of Medical Research*—Since 1913 the Indian Journal of Medical Research printed under the aegis of the Indian Council of Medical Research (formerly Indian Research Fund Association) has been edited by the Director, C.R.I., Kasauli. It contains original research papers on bacteriology, virus diseases, pathology, pharmacology, biochemistry, nutrition and public health engineering. Up to 1957, the Journal appeared quarterly but from January, 1958 it is being published once in two months (six issues in a year) and has attracted papers from research workers in India and abroad.

Till 1958, the distribution, sale, maintenance of accounts, booking of advertisement and allied duties were performed by the publishers, but from January, 1959 all functions, except printing have been taken over by the Editorial Office. The Journal has 450 subscribers in India and abroad. In addition, 130 journals were received in exchange for the *Indian Journal of Medical Research.*

In July, 1961 a special number of the Journal consisting of 228 pages was brought out to commemorate the Golden Jubilee of ICMR. This issue contained articles from well-known research workers in India and abroad.

In September, 1961 the business office of the journal was shifted to Delhi and is located in the building of ICMR. The Editorial Office continues to be located at Kasauli. It is proposed to bring out 12 issues of this journal in future.

4. *Indian Council of Medical Research Microfilm and Photo Service Unit*—The Microfilm and Photocopy Unit of the Indian Council of Medical Research was established at this Institute in 1948. Since then it is supplying at nominal cost, microfilm and photocopies of references available in the two libraries housed at this Institute.

During the year under review, the unit supplied 919 pages of microfilms, 832 pages of photocopies and 21 laboratory photographs with 224 photoprints to about 90 individuals and Institutions working in the field of medicine and allied sciences.

IX. MISCELLANEOUS

1. *Semple Scientific Society*—The following talks were arranged on scientific and technical subjects under the auspices of the Semple's Scientific Society, during the year 1962—

6th July, 1962

Dr. A. Guha, D. Phil. (Science), Reader and Incharge of Biophysics Department, College of Medical Sciences, Banaras Hindu University—"Molecular aspects of Bacteriophage multiplication".

9th November, 1962

Dr. (Miss) N. F. Millis of Melbourne University, Australia—"Citric Acid Fermentation".

20th November, 1962

Dr. F. Kornalik, M.D., C.S. of Institute of Experimental Pathology, Charles University, Prague—"Snake Toxins with special regard to blood coagulation".

11th December, 1962

Dr. S. C. Agarwal, M.D., Ph.D., Assistant Director, C.R.I., Kasauli—"Chloromycetin Sensitivity and Phage Lysis of Salmonella species in our country".

2. *Institute Common Welfare Fund*—The fund is registered with the Punjab Government as a Charitable Fund. It has gained much popularity among the members of the staff.

Loans and grants in cases of illness and other welfare activities of the members of the staff were sanctioned interest free. Out of Rs. 1,000 sanctioned as grant-in-aid by the Officers Fund wool worth Rs. 500 has been purchased for making woollen garments for the soldiers.

In emergencies the employees are granted advance of pay which is recovered in full on next pay day. About one hundred employees have been given help in the shape of loans. The fund has now assets of about Rs. 3,100.

3. *Nari Kalyan and Shishu Kalyan Kendra*—The kendra has gained much popularity among the staff members. The children of the employees are given free skimmed milk and iron tablets received from the UNICEF and Red Cross Society, respectively. About 100 children are being benefited daily by the kendra. The milk is distributed under very hygenic conditions.

The Kendra takes keen interest, as usual, in celebration of National Days.

The wives of the Officers and staff of the Institute have undertaken to knit woollen garments for the soldiers and took great interest in other activities of the Kendra.

4. *C.R.I., Sports Club*—The C.R.I. Sports Club continued its varied activities in the out-door and in-door games in spite of the fact that the local military ground was not available as during the previous year. The various teams of the club played friendly matches both in station and out-station.

5. *Recreation Room C.R.I. Kasauli*—The recreation room is provided with a radio set for the entertainment of the staff during lunch hour and after working hours. Indoor games are also played in the recreation room.

As in the previous years a number of documentary and educational films were shown to the Institute staff and their families.

On the two National Days viz. Republic Day and Independence Day, after flag hoisting ceremony and singing of National Anthem by the Staff of the Institute, recreational games were arranged for the children of the staff and tea and snacks served to the employees. Sweets were also distributed to the children of the employees.

6. *C.R.I. Co-operative Credit and Thrift Society Ltd. Kasauli*—The society is registered with the Punjab Co-operative Department since 5th May 1925. The main objects are to promote economic interests of its members, to help members to tide over financial stringencies, keeping in view the spirit and practice of thrift and mutual self help. The Society also runs a Co-operative Store for the benefit of the employees.

The license of the Society to sell wheat and food grains during the year was renewed from the Assistant Food and Supplies Officer, Simla. The Society is already in possession of a license for the sale of drugs and medicines.

Stores and chemicals amounting to Rs. 24,240.21 nP. were sold and loans of Rs. 11,193.85 nP. were advanced to the members during the year.

The Society has already purchased twelve Years National Plan Savings Certificates worth Rs. 5,000.

The running capital of the Society is Rs. 38,402.87 nP. and it has 230 members on its roll.

X. STATISTICS OF ANTIRABIC TREATMENT FOR THE YEAR 1961

Type of vaccine—The vaccine employed at Kasauli and at the treatment centres supplied with Kasauli vaccine, is a 5 per cent emulsion made from the brain of sheep infected with Paris strain of fixd virus.

During the year 1961, 52,531 patients received full treatment at the Institute and Centres, as compared with 15,872 in 1960; 56,085 in 1959; 55,519 in 1958; 56,515 in 1957; and 55,000 in 1956.

The total number of patients attending the Institute and its centres is as follows:—

	1961
Fully treated	52,531
Partially treated	28,588
Advice cases	10,352
Cases excluded from statistics for various reasons	208
Total ..	91,679

Partially treated are those who commence treatment but do not complete the full course. The ratio of these to those fully treated is almost 1:2.

Advice cases are those who come for treatment but who are not considered at risk these form 11.29 per cent of the total attending.

The antirabic treatment centres supplied with vaccine numbered 321, *viz.*—

Punjab	73
Jammu & Kashmir	11
Military	87
Others	150
Total ..	321

The statistical data relating to cases of antirabic vaccine treatment are presented in Appendix 1 to 2 and Tables I to VI.

Out of the total of 52,531 fully treated cases, 5 deaths from hydrophobia were reported. Death rate amongst cases treated at the Institute and its centres was 0.009 per cent.

After effects of treatment—

General effects—Complaints having no casual relationship to antirabic treatment were received from some of the treatd patients, while three cases of paralytic accidents were reported during the year, details of which are as follows:—

1. Case No. 34, male, boy, aged 17 years, commenced antirabic treatment on 23rd January 1961, at Civil Hospital, Bassi Pathanan, and received 10 injections of 10 c.c. each of antirabic vaccine. On 13th February 1961, the patient developed weakness and retention of urine. On 7th March 1961, the Assistant Surgeon, Bassi Pathanan, reported that the patient could pass urine himself, and is improving gradually.

2. Case No. 13, male, adult, aged 23 years, commenced antirabic treatment on 26th May 1961 at M.I. Room, Military Hospital, Jhansi, and received 7 injections of 2 c.c. each of antirabic vaccine. The patient started neuroparalytic symptoms such as retention of urine, weakness of lower limbs etc. 8 days after commencement of treatment. The Officer Commanding, Military Hospital, Lucknow, reported that the patient has slight weakness and his neurological condition has improved on the whole.

3. Case No. 161, male, boy, aged 9 years, commenced antirabic treatment on 4th August 1961, at General Hospital, Banswara, and received 14 injections of 2 c.c. each of antirabic vaccine. After 22 days the patient developed symptoms of encephalomyelitis and died on 8th September 1961, the same day at the hospital.

STATISTICS OF ANTIRABIC INOCULATIONS

Appendix I

The following compares the numbers of persons fully treated with antirabic vaccine, the number of deaths and the death rates from hydrophobia among these persons with the registered deaths from hydrophobia reported in Punjab for the year 1961—

Year	Province	Total Population	Total treated	Deaths from hydrophobia amongst treated cases	Death rate, per cent	No of registered deaths from hydrophobia (untreated)
1961	Punjab	20,300,912	27,072	4	0 014	98

Appendix II

Addendum to the Report of the Central Research Institute, Kasauli, for the year 1960 to complete the records

After the figures for the report for 1960 were completed, the undermentioned death from hydrophobia amongst cases treated at Central Research Institute and its centres was reported—

Serial No.	Case number	Number of days from commencement of treatment to onset of symptoms	Sex	Age, years	Biting animal	Days late in commencing treatment	Duration of illness, days	Interval between bite and death, days	Class of case	Duration of treatment, days
1	158	26	Male	12	Dog	1	3	30	II (14×10cc)	14

TABLE I

A general classification of patients attending the Institute and its Centres

	Class of cases treated		
	I	II	Total
Number fully treated	6,858	45,673	52,531
Percentage to total	13·06	86·94	..
Number partially treated	..	..	28,588
Percentage to grand total	..	..	31·18
Total deaths amongst treated	..	5	5
Total deaths amongst fully treated ..	..	3	3
Deaths rate per cent. amongst fully treated ..	..	0·006	0·005
Percentage of health returns	45	47	47
Advice cases, not at risk	..	..	10,352
Cases excluded from statistical for other reasons	..	..	208
Grand Total ..	..	..	91,679

TABLE II

Deaths from hydrophobia amongst persons—Institute and Centres, 1961

Serial No.	Case No.	Centre	Sex	Age years	Biting animal	Days late in commencing treatment	Number of days from commencement of treatment to onset of symptoms	Duration of illness days	Interval between bite and death, days	Class of case and type of treatment	Duration of treatment, days
1	336	Civil Hospital, Gurgaon	Male	19	Dog	10	110	1	130	II (14×5 c.c.)	14
2	1,139	Civil Hospital, Rohtak	Male	8	Dog	2	13	..	15	II (14×5 c.c.)	10 (Incomplete treatment).
3	238	Civil Hospital, Muktsar	Male	16	Dog	30	25	2	57	II (14×5 c.c.)	14
4	249	M.B.H. Abohar ..	Male	25	Dog	..	209	3	212	III (14×10 c.c.)	14
5	..	Hindu Rao Hospital, Delhi	Male	50	Dog	2	20	1	23	III (14×10 c.c.)	13 (Incomplete treatment).

TABLE III

A general statement regarding patients treated at the Institute and its Centres from 1900 to 1961

Year	Europeans and Asiatic combined			
	Number treated	Deaths	Death rate per cent	Advice
Total 1900 to 1960	10,65,389	3,452 (One death shown in addendum in the annual report 1961).	0·32	74,381
1961	52,531	5	0·009	10,352
Total 1900 to 1961 ..	11,17,920	3,457	0·309	84,733

TABLE IV

Classification by the animals which bit, scratched or were in contact with patients—Institute and its centres 1961

Serial No.	Animals	Number treated	Percentage to total	Death rate per cent
1	Dog	5'18,793	92·88	0·01
2	Jackal	565	1·07	
3	Human	812	1·54	
4	Cow, Calf, Bullock, Ox	913	1·73	
5	Cat	55	0·1	
6	Monkey	158	0·3	
7	Donkey, Ass	69	0·13	
8	Buffalo	754	1·43	
9	Wolf	11	0·02	
10	Mongoose	134	0·25	
11	Mule	4	0·007	
12	Hyena	9	0·01	
13	Goat	82	0·15	
14	Fox	15	0·02	
15	Camel	37	0·07	
16	Leopard, Panther	13	0·02	
17	Bear	3	0·005	
18	Badger	1	0·001	
19	Horse, Mare, Pony	71	0·13	..
20	Tiger	3	0·005	..
21	Lion	5	0·008	..
22	Pig	22	0·04	..
23	Vulture	1	0·001	..
24	Unknown	1	0·001	..
	Total ..	5/52,531	..	0·09

TABLE V

Statement showing the number of brains examined for rabies at the Central Research Institute, Kasauli, during the year 1961

Serial No.	Name of specimen	Number of brains received	Microscopically			Biologically		
			Positive	Negative	Unfit for examination	Positive	Negative	Unfit for examination
1	Dog brains	142	66	64	8	19	39	19
2	Cat brains	3	1	2	..	..	1	1
3	Buffalo brains	1	..	1	..	..	..	2
4	Calf brains	3	1	1	1	..	1	..
5	Cock brains	1	..	1	..	..	..	1
6	Bullock brains	1	..	..	1	..	..	
	Total ..	151	68	69	10	19	41	24

TABLE VI

Classification according to the animals which bit, scratched or were in contact with patients from 1908 *to* 1961

Serial No.	Animals	Number treated	Percentage to total	Deaths	Percentage death rate
1	Dog	5,9,84,900	88 52	2,460	0·24
2	Jackal	70,747	0·35	893	1·26
3	Human	14,968	1·34	2	0·01
4	Cow, Calf, Bullock, Ox ..	11,519	1·03	..	..
5	Cat	2,702	0·24	2	0·07
6	Monkey	7,393	0·66	..	..
7	Donkey, Ass	2,035	0·18	..	..
8	Buffalo	7,451	0·66	..	..
9	Goat	1,129	0·101	..	..
10	Horse, Mare, Pony	3,480	0·31	..	..
11	Pig, Boar	346	0 03	..	..
12	Mongoose	1,836	0·16	2	0·1
13	Wolf	1,004	0·09	65	6·47
14	Mule	311	0·02	..	..
15	Hyena	492	0 04	16	3·25
16	Otter	85	0 007	..	..
17	Fox	217	0·01	1	0·46
18	Camel	504	0·04	..	..
19	Leopard, Panther.. ..	414	0·03	9	2·17
20	Bear	69	0·006	..	
21	Rabbit	28	0·002	..	
22	Eagle	1	..	..	
23	Badger	55	0·004	..	
24	Rat	99	0·008	..	
25	Kite	2	..	..	
26	Sheep, Ram	49	0·004	..	
27	Tiger	314	0·02	..	
28	Elephant	2	..	..	
29	Guinea-Pig	1	..	..	
30	Lion	39	0·003	..	
31	Miscellaneous	10	..	..	
32	Squirrel	16	0·001	..	

TABLE VI—*contd.*

Serial No.	Animals	Number treated	Percentage to total	Deaths	Percentage death rate
33	Laboratory infection	16	0·001	..	..
34	Goose	4	..	..	..
35	Vulture	12	0·001	..	..
36	Cock, Hen, Fowl	3	..	..	..
37	Hawk	5	..	..	..
38	Owl	1	..	..	..
39	Sow	2	..	..	..
40	Duck	5	..	..	..
41	Blackapc	5	..	..	..
42	Deer	17	0·001	..	..
43	Rodent	3	..	..	..
44	Zebra	1	..	..	..
45	Mouse	16	0·001	..	..
46	Unknown	324	0·02	7	2·16
	Total ..	11,12,611	..	3,457	0·3